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CLINICAL AND EXPERIMENTAL

THE METEOROTROPISM OF ECLAMPSIA

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THE influence of meteorologic changes on the outbreak and course of human diseases has long been acknowledged and for decades medicine has sought to explain this correlation. In this respect, perhaps eclampsia has been most frequently investigated, but although a large number of European workers have written regarding its relationship to changes in the weather, it has received less attention in the United States.

Only since a common basis of explanation for meteorologic factors has been found and made relatively easy to understand have the studies regarding the meteorotropism of eclampsia and other diseases received a new impetus. A short description of modern meteorologic conceptions is necessary before going on to a discussion of eclampsia and its relation to weather changes. De Rudder in Germany has written a book on meteorobiology of the human being, giving the medical man a concise explanation of the new concepts. Petersen's monographs *The Patient and the Weather* present a more exhaustive treatment of the same subject.

The most important cause of weather change is the passage of the so-called discontinuity surface or front which separates air masses of opposite characteristics. The two general types of air masses operating in this system are the polar air masses originating over the polar and the subpolar regions and the tropical air masses originating over the subtropical zone. The main differences between these air mass types when of continental origin are as follows:

	POLAR CONTINENTAL	TROPICAL MARINE
Temperature	Cold	Warm
Humidity	Dry	Humid
Permeability to sunlight	Relatively transparent	Relatively opaque
Permeability to short wave rays	Strong	Poor
Ion content	High	Low
Electrical potential (Spannung)	Low	High

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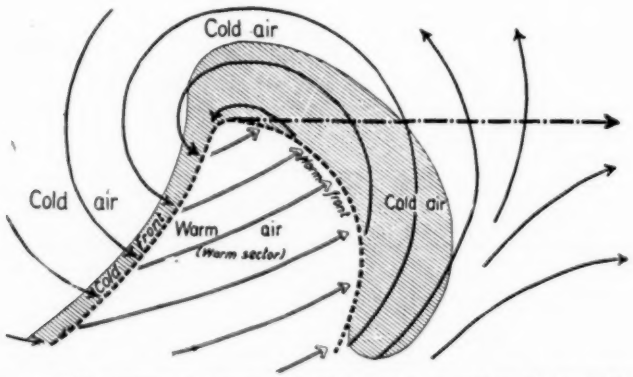

Fig. 1*a*.—The passage of warm and cold fronts as projected by Bjerknes.

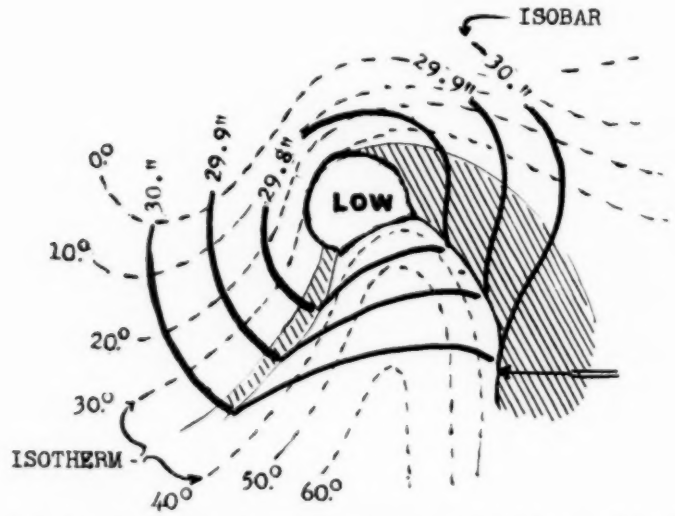

Fig. 1*b*.—The relationship of theoretical fronts to the isotherms and isobars.

Since these air masses mix to no appreciable degree, we generally find the discontinuity surfaces to be zones of rapid transition of the meteorologic elements. These regions of rapid transition are termed "fronts." The cold front is the discontinuity between the tropical current and the advancing wedge-shaped polar air mass. This wedge of cold air forces the warm air aloft, giving rise to clouds and precipitation. Often, too, the cold front is a rather turbulent affair, especially in the winter months. As we have indicated, the cold front is marked by a sharp drop in the temperature, a clearing of the air in a few hours and lowering of the humidity, and a steady rise in the barometric pressure. Generally the wind shifts from the southwesterly direction of the tropical current to the northwesterly direction of the polar current. The warm front, on the other hand, is the discontinuity surface between the receding modified cold air and the tropical current. The warm front is generally a more gradual transition than the cold front, and it is marked by increasing temperature and

humidity and a falling barometer. We shall refer to the period of weather from cold front to warm front as the "polar episode," and the period of weather from the warm front to the cold front as the "tropical episode." These two periods need not contain "pure" polar or tropical air masses, but they have been so labeled for convenience.

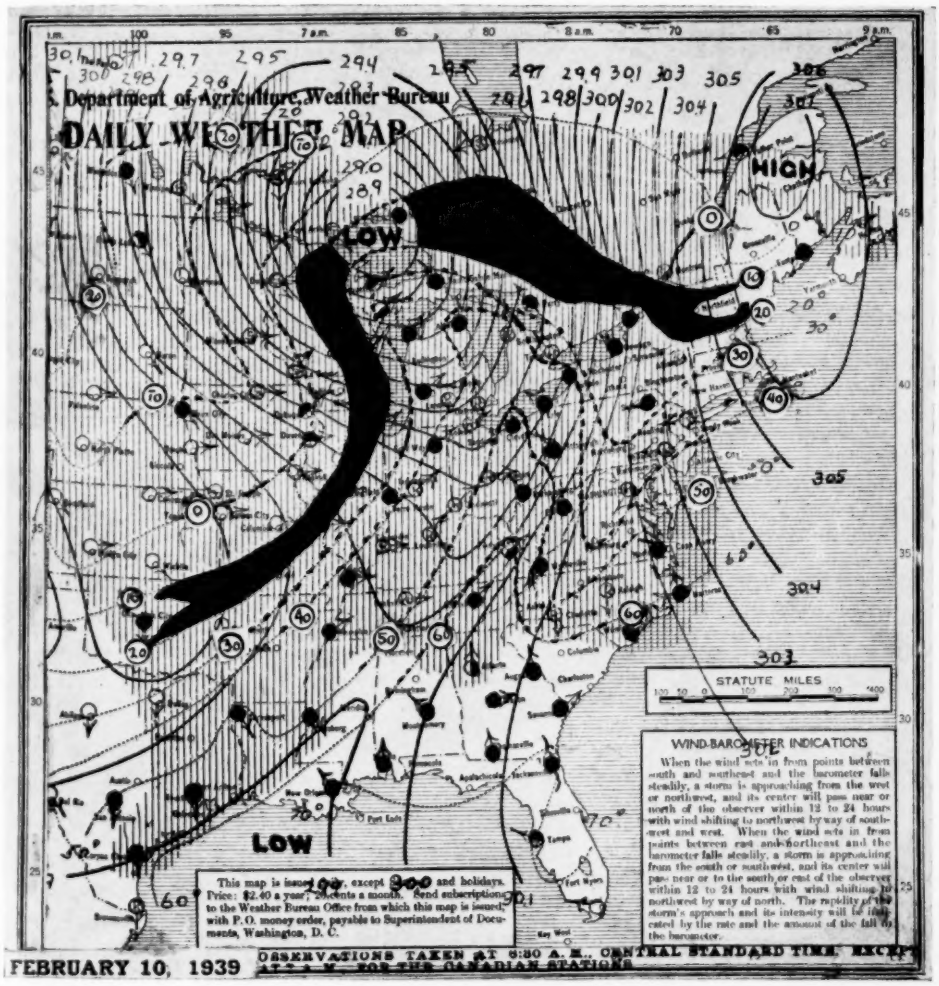
Department of Agriculture, Weather Bureau
DAILY WEATHER MAP
HIGH
LOW
LOW
STATUTE MILES
WIND-BAROMETER INDICATIONS
When the wind sets in from points between south and southeast and the barometer falls steadily, a storm is approaching from the west or northwest, and its center will pass near or north of the observer within 12 to 24 hours with wind shifting to northwest by way of southwest and west. When the wind sets in from points between east and northeast and the barometer falls steadily, a storm is approaching from the south or southwest, and its center will pass near or to the south or east of the observer within 12 to 24 hours with wind shifting to northwest by way of north. The rapidity of the storm's approach and its intensity will be indicated by the rate and the amount of the fall of the barometer.
This map is issued daily except Sundays and holidays. Price: $2.40 a year; 20 cents a month. Send subscriptions to the Weather Bureau Office from which this map is issued, with P.O. money order, payable to Superintendent of Documents, Washington, D. C.
FEBRUARY 10, 1939
OBSERVATIONS TAKEN AT 8:30 A.M., CENTRAL STANDARD TIME, EXCEPT AT ★ W FOR THE CANADIAN STATIONS

Fig. 1c.—An actual frontal passage as reflected in a typical weather map. The episode is for February 10, 1939.

The frontal system is arranged in "depressions" or areas of low barometric pressure according to the idealized schemes outlined in Fig. 1, *a*, *b*, *c*.* It must be emphasized that these diagrams are idealistic and that the daily analysis of the weather is by no means such a simple affair. This low pressure area moves generally from west to east, accompanied by most of our weather changes. There are, however, local weather changes that might also be concerned in the course of

*The interested reader is referred to Horace Byers' *Synoptic and Aeronautical Meteorology*, New York, 1937, for a more detailed discussion of the modern point of view of air mass analysis.

disease, but in this paper we are interested only in the effect of weather changes —frontal passages—on the health of the maternal population.

The first studies concerning eclampsia and weather built upon the shortly characterized new meteorologic conceptions were carried out by Jacobs and von Heuss, who finally concluded that eclampsia follows only the infall of cold air. Von Latzka in Budapest came to the same conclusion. Eufinger and Weikersheimer, Ponzi, and others believe that the passing of warm fronts can have the same effect in producing convulsions, but in a considerably lower percentage. In a recent paper by Louros and Panajotou in Greece, the prevalence of the cold front influence, as described by Jacobs, is denied, and the "change of air masses" in either direction is emphasized as a cause for the outbreak of eclamptic convulsions.*

Before going into our own studies on this question, it is necessary to consider one main difficulty concerning the reaction time elapsing between the meteorologic event and the onset of the eclampsia. When air mass changes follow each other at short intervals, which happens to be the case in Chicago where our material was collected, it is often not easy to decide if an eclamptic convulsion is associated with the passing of a warm front or with the invasion of cold air shortly preceding it. Jacobs, who acknowledges only a cold air invasion as the precipitating cause, still inculpates it even if there has been a definite passage of a warm front between the occurrence of the cold front and the onset of the convulsions. De Rudder states, with truth, that there is no reason why the warm front should not be acknowledged equally, because the main problem to be solved is whether *any* weather change takes place at the same, or approximately the same, time as the outbreak of the disease. A further difficulty consists in the fact that warm fronts are generally more gradual transition periods than cold fronts. The accurate determination of warm fronts, therefore, is considerably more difficult. Further, the fact may be stressed that the weather does not entirely consist of a series of abrupt air mass changes, but often the passage of fronts is so slow and diffuse that the real change of air mass is not so obvious.

Of course it would be ideal, as Dieckmann states in his recent paper, if one could collect the records of all toxemic pregnant patients for a city of several million people for comparison with the meteorologic data over the same period. Even then it would be extremely difficult to clarify the relations, because only the outbreak of convulsions is a clinical event definite enough to be reported with the exact time of its occurrence, and only these exact data are usable. (Since the prophylactic treatment of pregnancy toxemia has been steadily improving, convulsions occur more and more infrequently.) To deviate a moment: Exact data of this kind have been gathered in various other diseases which, like

*The confusion may arise from the fact that with warm weather the patient becomes more alkaline, and under such conditions minor environmental alterations or disturbances may cause vascular spasm and initiate an eclamptic convulsion. This is possibly the reason for the curious distribution of eclamptic deaths in the United States, to which Petersen called attention. Petersen assumes that vascular spasm is the underlying factor and has analyzed the mechanism as an autonomic dysintegration, most often precipitated by weather disturbance in susceptible individuals. (Petersen, William F.: *The Patient and the Weather.* Vol. II, Autonomic Dysintegration, Ann Arbor, Mich., 1934, Edwards Brothers; Vol. IV, Part 1, Cardio-Vascular-Renal Disease, 1936.)

eclampsia, are closely connected with the vascular system and are precipitated by a change of tonus controlled by the vegetative nervous system. This change in tonus, for instance in apoplexy, acute onset of glaucoma, acute laryngeal croup, spasmophilia, etc., has been definitely proved to follow a frontal passage.

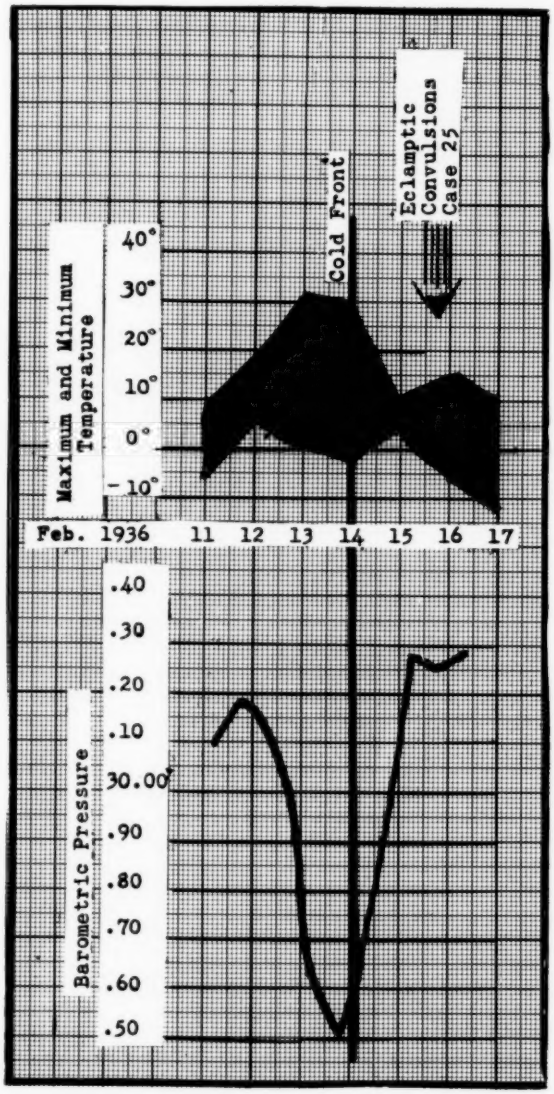

Fig. 2.

Out of a material of 425 cases of toxemia of pregnancy collected by Dr. F. H. Falls* in the Cook County Hospital and the Research and Educational Hospital, University of Illinois, College of Medicine, Chicago, during the years 1931-1937 for other purposes, one of us (F.) chose 46 cases for this study. All these were eclampsia with convulsions. The high number of pre-eclamptic and toxemic patients could not be included for the simple reason that, as previously

*Dr. Falls was kind enough to allow a study of his cases.

pointed out, the connection between weather and eclampsia primarily concerns the precipitation of convulsions. The clinical records of the 46 cases included the most exact data concerning the time of the outbreaks in hours and minutes. The corresponding weather charts were kindly provided by Dr. W. F. Petersen. Further, the official daily weather reports of the U. S. Weather Bureau were at our disposal.

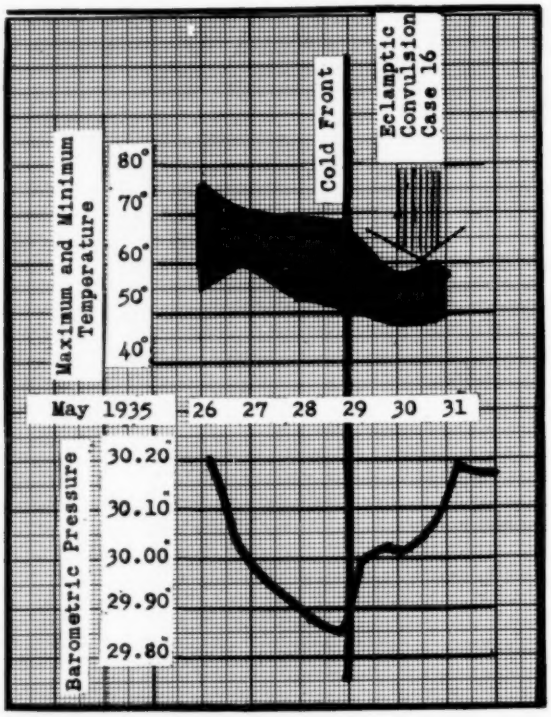

Fig. 3.

The 46 cases studied concerned 36 white and 10 colored patients. Thirty-one, or 67.4 per cent, were primiparas; 15, or 32.6 per cent, multiparas. Of the 36 white patients, 24, or 66.6 per cent, were primiparas; 12, or 33.4 per cent, multiparas; of the 10 colored, 7 or 70 per cent, were primiparas; 3, or 30 per cent, multiparas. The age distribution was as follows:

Below 20 years, 12 = 26 per cent
20 - 30 years, 17 = 37 per cent
30 - 40 years, 11 = 24 per cent
Over 40 years, 6 = 13 per cent

In the group between 30 and 40 were 2 primiparas. In 21, or 45.7 per cent, the eclamptic convulsions broke out ante partum; in 13, or 28.3 per cent, intra partum; and in 12, or 26 per cent, post partum. The percentage of post-partum eclampsia seems remarkably high.

Delivery was spontaneous in 15 cases or 42.9 per cent; labor was induced in 6, or 17.1 per cent; cesarean section was performed in 8, or 22.9 per cent; vaginal operations (forceps, version, and extraction) were carried out in 6, or 17.1 per cent. In 12 cases, as already mentioned, eclampsia broke out only after delivery.

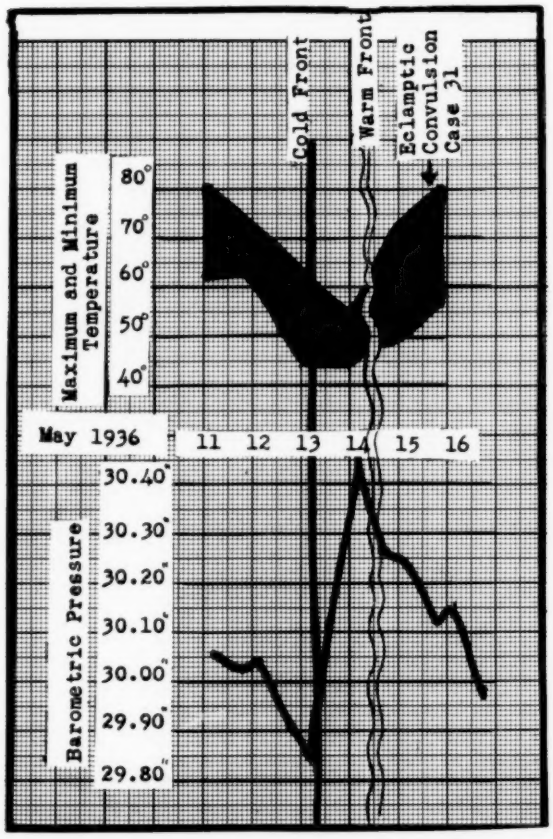

Fig. 4.

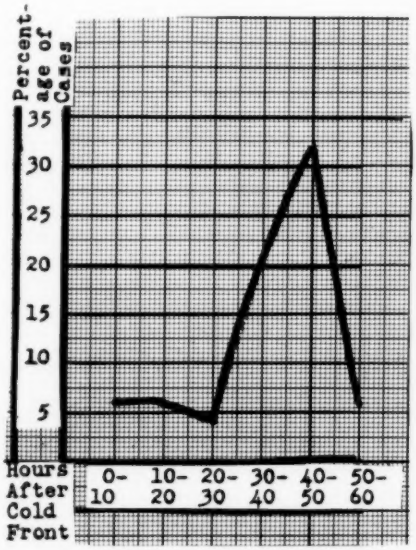

Fig. 5.

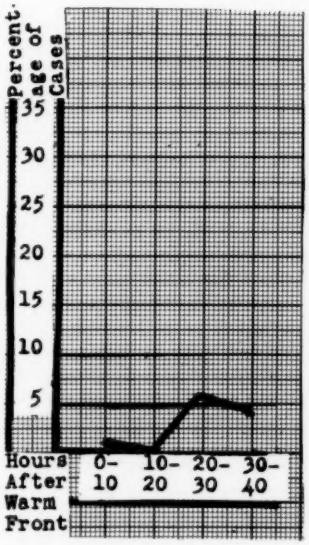

Fig. 6.

The fetal mortality was 10.8 per cent (5 cases), the maternal mortality 6.5 per cent (3 cases). The low percentage of fetal and maternal mortality speaks for the greatly improved treatment available. The number of observed convulsions ranged on an average between 1 and 9; in one case which ended well, no fewer than 54 convulsions occurred.

As far as the seasonal distribution is concerned, the highest number of cases was observed in April, as noted by Harrar and Davis in New York in 1926. A renewed increase in cases was found in August. In statistics concerning 36 cases of eclamptic death in 1932 in Chicago, the highest numbers were found in January, March, and October. Dieckmann reported different months of prevalence from the different regions of the earth. All these findings suggest that the season in itself is not associated with the disease, but the singular weather episodes which can be absolutely different in different years.

In ascertaining the association between the 46 eclampsia cases and changes of air masses, we obtained the following results: In 34, or about 74 per cent, of the convulsions were connected with infalls of cold air; in 6 cases, or 13 per cent, with passing of warm fronts. Figs. 2, 3, and 4 show how this was worked out. In the remaining 6 cases, or 13 per cent, the weather was too unsettled to permit definite conclusions. In 2 of these cases we are inclined to consider a polar front, and in 4, a warm front passage to be the factor. The following data refer to the interval between the meteorologic event and the outbreak of the convulsions:

0 - 10 hours after cold front:	3, or about	6 per cent
10 - 20 hours after cold front:	3, or about	6 per cent
20 - 30 hours after cold front:	2, or about	4 per cent
30 - 40 hours after cold front:	9, or about	20 per cent
40 - 50 hours after cold front:	14, or about	32 per cent
50 - 60 hours after cold front:	3, or about	6 per cent
Total during "polar episode"	34, or about	74 per cent

0 - 10 hours after warm front:	1, or about	2 per cent
10 - 20 hours after warm front:	0, or about	0 per cent
20 - 30 hours after warm front:	3, or about	6 per cent
30 - 40 hours after warm front:	2, or about	4 per cent
Total during "tropical episode"	6, or about	13 per cent

The curves in Figs. 5 and 6 illustrate these data. Thus the evidence shows that much the highest percentage of cases are distinctly correlated with the passing of a cold front and that the highest number of onsets occur within forty and fifty hours after the invasion of cold air.

As previously stated, our study of the association between weather changes and eclampsia has been confined to the precipitation of convulsions. We have no intention of claiming that the weather has any etiologic significance with regard to the disease itself. The etiology of eclampsia is, in spite of the existing large number of old and new theories, still an unsolved problem. The only fact beyond any doubt is that it consists in a severe metabolic disturbance of the maternal organism. As far as the mechanism of the precipitation of eclamptic convulsions is concerned, one can conclude that some factor influencing the vegetative nervous system of the toxemic or pre-eclamptic patient plays the main role. It is easily understood that sudden changes in the physical milieu produce these vegetative disturbances which can be counterbalanced by the normal

healthy organism, but not by the metabolically disturbed organism. Haselhorst, Hinselmann, Nevermann, and Heynemann proved that the lability of the capillaries is increased in eclamptic patients. Petersen states that weather changes precipitate even in the normal organism definite alteration in metabolism. The results of his extensive research in this matter show that cold air invasions precipitate contraction of the skin capillaries, and a rise in blood pressure; further, hyperglycemia, a relative alkalosis, and a fall in the carbon dioxide tension in the blood. He expresses the sum of all the physiologico-chemical changes precipitated by a cold front as the ARS phase (A = anabolism, R = reduction, S = spasm). Opposite physiologico-chemical effects occur with the passage of a warm front. They are designated by Petersen as the COD phase (C = catabolism, O = oxidation, D = dilatation).

It is possible that electrical changes which occur in the atmosphere during the passage of a front influence the labile organism of the toxemic pregnant patient. Elster and Geitel (quoted by de Rudder) have described the so-called "ions of the atmosphere." They consist of electrically loaded particles which are suspended in the air. Louros and Panajotou agree with Piéry in France who states that these ions have definite influence upon the lability of the vegetative nervous system. Düll and Düll have sought to associate solar eruptions, which cause electrical magnetic disturbances upon the earth. These solar eruptions are, in their opinion, responsible, not only for the atmospheric occurrences, but also for the reaction of the living organisms. At present we are only just entering this new field of research, but one can already foresee that highly interesting results will be obtained in the near future.

Even from the practical point of view the conclusions obtained are of some value. It is interesting that in a recently published pamphlet on *Weather and Season in Surgery*, by Maurer, a certain number of prophylactic and therapeutic measures are recommended in consideration of the association between weather and disease. As early as 1932, Kümmel recommended that major surgery, with the exception of urgent cases, should not be performed during frontal weather. Similarly, it should be possible to work out some practical prophylactic measures against the precipitation of eclamptic convulsions through weather changes. A well-organized service for "prenatal care" should pay attention to the weather conditions. Imminent front passages are known to the official Weather Bureaus early enough to be communicated to the different prenatal services. Known toxemic or pre-eclamptic patients could be especially examined at these danger periods and proper prophylactic treatment could be administered. In this way the number of cases with convulsions could be further diminished.

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STUDIES IN CELLULAR EXUDATES OF BOWEL DISCHARGES*

I. Control Observations in 1,123 Patients, 7 Autopsies, and 3 Dog Experiments

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INTRODUCTION

THE diagnosis of pathologic conditions of the bowel can be simplified by an understanding of the significance of cellular exudates in bowel discharges. Early work establishing the differential diagnosis of amoebic and bacillary dysentery on a basis of the cellular exudates in the diarrheal discharges in those two conditions has led to the wider study of cellular exudates in bowel discharges in order to determine their importance as a diagnostic procedure.

The studies which are being reported in this series include control observations in so-called normal individuals, that is, in those who state that they have no bowel complaints of any sort, and autopsy findings in man and in the dog. In the near future the series will be supplemented by the presentation of observations, now in process of completion, on the various cytologic pictures that are to be found. The results will be given of cellular exudate studies in patients with various types of bowel complaint. It is hoped that this work will make it possible for pathologic change in the bowel to be diagnosed with a greater degree of accuracy than has been practicable hitherto.

HISTORICAL

The diagnostic significance of cellular exudates in bowel discharges has long been recognized. Dutcher[1] was a pioneer in the field, and his contributions to the subject made as far back as 1903 (referred to by Bahr[2])

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were followed by the early work of Jurgens.[3] In 1910, during an epidemic of dysentery in Fiji, Bahr demonstrated the practical value of the microscopic examinations of the stools for cells. Renewed interest in the subject came in 1917 when Bartlett[4] made a report on the pathologic aspects of dysentery from which the British Mediterranean Expeditionary Forces were suffering. He regarded amoebic dysentery as the major factor in the depletion of the ranks of the soldiers. Bahr and Willmore[5] replied to Bartlett's report in detail and showed that many cells were of tissue origin and were not *Endamoeba histolytica*, as Bartlett had asserted. They acknowledged that these cells could be mistaken for amoebae very easily and this error was responsible for much confusion in differential diagnosis. Wenyon and O'Connor[6] independently investigated the same problem and reached the same conclusions as Bahr and Willmore, but intimated that they thought the cellular exudate studies of bowel discharges might be of value in the diagnosis of bowel conditions other than amoebic and bacillary dysentery. Subsequent writers, including Graham,[7] Willmore and Shearman,[8] Anderson,[9] and later, Callender,[10] Haughwout,[11] and others, have made outstanding contributions in confirmation of the earlier work done in the field. As a result of these investigations, it is now an accepted fact that there are definite cytologic pictures in amoebic and bacillary dysentery and that these can be differentiated. The success which has attended the work on dysentery has stimulated inquiry into the relationship of cellular exudates to ulcerative colitis and to other types of bowel complaints. The early suggestion of Wenyon and O'Connor has been amply confirmed in the investigations herewith reported.

The special studies on cellular exudates, which have been carried out during the past four years, have established the fact that whenever pathologic change takes place in the bowel mucosa, cells of various types will be found in the discharge. Conversely also, when no cells are found in the bowel discharge, no pathologic condition is present. The presence or absence of these cells can be determined readily by microscopic examination of the bowel discharges. While this method of examination is one of the simplest, it is also one of the most effective available for the diagnosis of pathologic conditions of the bowel.

CELLULAR EXUDATES AND THE NORMAL BOWEL

A series of observations was made to determine first of all whether or not cellular exudate would be found in the discharge of patients whose bowel functions were reported to be normal. In order to establish a reliable check upon these observations, a series of autopsy examinations was also made. The control observations were made on a group of 1,123 patients selected at random from the venereal disease clinic at the Meinhard Health Center of the Department of Health of the City of New York. These patients were studied as part of an extensive survey which is being carried out by the Parasitology Service of the Bureau of Laboratories, Department of Health, City of New York, John L. Rice, M. D., Commissioner. They were selected because on close questioning they stated that their bowel functions were absolutely normal and also because they affirmed that they had no complaints which could be referred to the gastrointestinal tract.

The specimens examined consisted of, first, the normally passed bowel evacuations and, second, the diarrheal discharge following dosage with Epsom salts. So that the freshly passed warm specimen could be studied, patients were asked to come to the laboratory to evacuate the bowels following the administration of the salts.

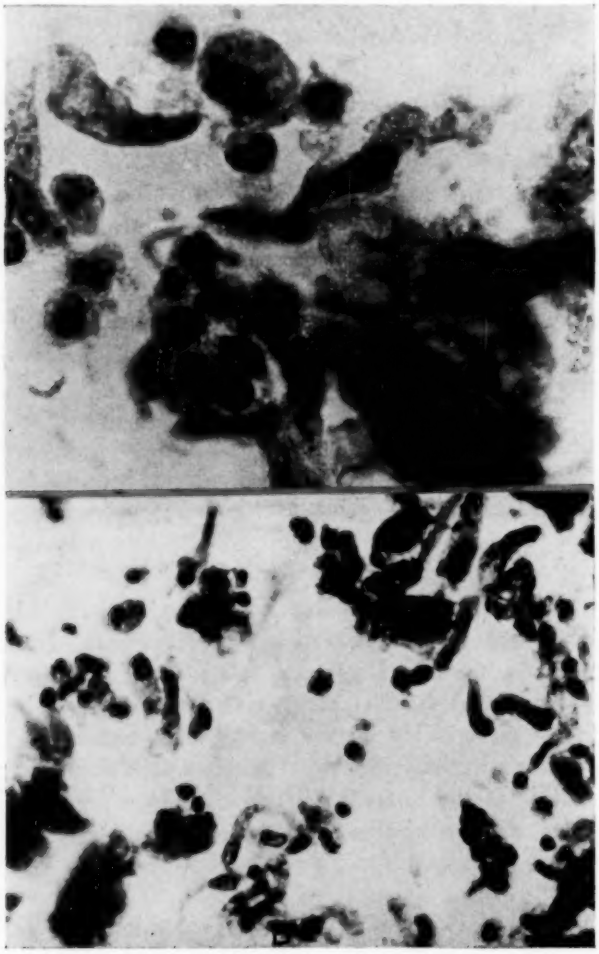

Fig. 1.—*A*, Degenerate cells found at autopsy in the bowel contents. Heidenhain's iron-hematoxylin stain. Oil immersion ×970; *B*, Cells found in scrapings from the bowel wall in the same patient at the same level as *A*. Heidenhain's iron-hematoxylin stain. High dry ×470. Compare with Fig. 2 cellular exudates from patients with chronic ulcerative colitis.

Human autopsy examinations were made through the courtesy of Dr. Auerbach, Pathologist at Seaview Hospital, and Dr. Ward J. MacNeal, Director of the Department of Pathology, New York Post-Graduate Hospital. Autopsy specimens included bowel contents and scrapings from several locations along the gastrointestinal tract. In each case scrapings from the bowel wall were taken from the same place as the bowel contents, and were placed in suitably marked bottles which contained 10 per cent formalin. This procedure made it possible to examine the fecal matter and the bowel wall to discover whether or not cells were present, and, if present, to compare the cells

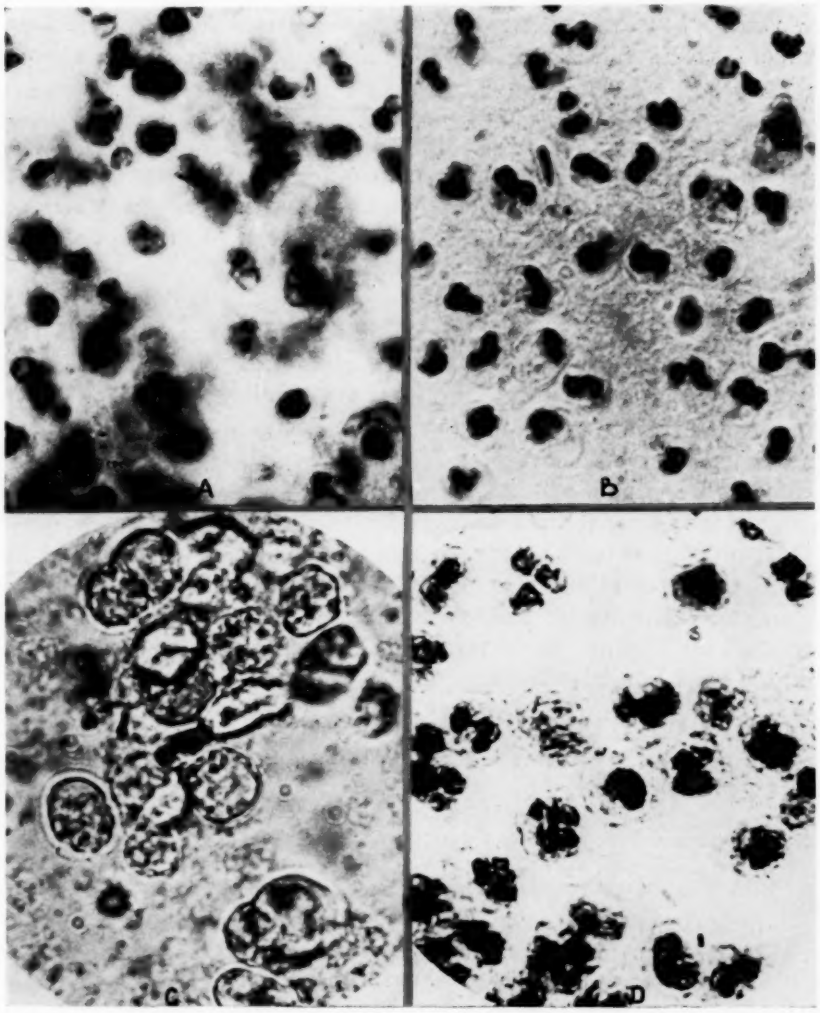

Fig. 2.—Cellular exudates from cases of chronic ulcerative colitis. Oil immersion ×970. *A* and *B* Heidenhain's iron-hematoxylin stain. *C* and *D* methylene blue.

found in each. It was exceedingly difficult to secure autopsy material that was fresh and had not undergone an excessive amount of post-mortem degeneration and sloughing. However, for the purpose of this study, which was to determine whether or not cells normally occurred in fecal matter, an investigation was made to discover whether or not the cells found in the contents corresponded exactly to those found in the scrapings from the same area. If so, it was assumed that those cells were probably a result of post-mortem changes rather than an actual cellular exudation from the bowel wall during life.

In order to deal with the problems raised by post-mortem degeneration, three dogs were given lethal doses of sodium amytal intravenously. Their abdomens were opened immediately and their bowels were studied before any post-mortem changes could possibly take place. In each instance, the blood had not even coagulated but flowed freely from the congested mesenteric vessels.

Specimens of the bowel contents in various locations in the colon, as well as scrapings from the bowel wall at those locations, were taken for study.

TECHNIQUE OF EXAMINATION

A wet cover slip preparation made up with methylene blue was used to prepare the freshly passed bowel specimens for examination. This technique proved to be the most satisfactory after an extensive study was made of several thousand microscopic examinations of specimens which had been prepared with many different dyes and by various methods.

A small portion of the bowel discharge should be taken on a wooden applicator (a toothpick or match will do) or on a platinum loop, and should be mixed with Loeffler's methylene blue on a glass slide and then covered with a cover slip. The specimen should not be allowed to dry at any time; otherwise, the cells will degenerate into an unrecognizable mass. Using the low-power magnification, the entire cover slip is examined minutely in order to determine the amount of cellular exudate. In order to study the cytologic structure, individual cells are examined with the high dry and oil immersion lenses.

Permanent preparations can be made by smearing a portion of the discharge onto a clean glass slide. This should then be fixed wet in Schaudinn's solution, and stained with Heidenhain's iron-hematoxylin technique, similar to that used in the study of protozoa. The technique used in the cellular exudate study is as follows:

Schaudinn's Solution

2 parts of saturated mercuric chloride solution
1 part absolute alcohol
5 c.c. glacial acetic acid to 100 c.c. of the above mixture

NOTE.—Prepare fresh each day before use.

Staining Method

1. Schaudinn's solution heated to 60° C. for 15 to 20 minutes.
2. Rinse in 50 per cent alcohol, then immerse successively into:
   70 per cent alcohol iodine, for 2 minutes;
   70 per cent alcohol, for 2 minutes;
   50 per cent alcohol, for 2 minutes.
   Rinse in water.
3. Mordant 2 to 4 per cent iron alum at 30° C. for 10 minutes (short 2 minutes).
4. Wash well in running water for 5 minutes.
5. Stain in 0.5 per cent Heidenhain's hematoxylin at 30° C., for 30 minutes (short 2 minutes).
6. Wash well in running water for 5 minutes.
7. Decolorize in 2 to 4 per cent iron alum.
   WATCH UNDER MICROSCOPE TILL NUCLEI ARE CLEARLY VISIBLE.
8. Wash well in running water for 5 minutes.
9. Immerse successively in 50 per cent alcohol for 2 minutes;
   70 per cent alcohol for 2 minutes;
   85 per cent alcohol for 2 minutes;
   95 per cent alcohol for 5 minutes;
   absolute alcohol for 5 minutes;
   absolute alcohol plus xylol for 5 minutes;
   xylol for 5 minutes.
10. Heat Canada balsam and mount.

In many instances it was necessary to preserve specimens in 10 to 20 per cent formalin. When this was done there was no loss of cells through degeneration. Examination of specimens preserved in formalin was undertaken over a period of six months, and with the methylene blue wet cover slip preparation it was possible to observe the cellular exudate without difficulty.

RESULTS

Table I gives the results of the examination of 2,158 specimens obtained from 1,123 patients who stated that they had no complaints which could be referred to the gastrointestinal tract. Nine hundred ninety-six patients presented 1,960 specimens of normal bowel evacuations. Epsom salts were given to 127 patients who came to the laboratory and passed 198 specimens of watery stools.

TABLE I

EXAMINATION OF SPECIMENS FROM PATIENTS WHO HAD NO COMPLAINTS REFERABLE TO THE GASTROINTESTINAL TRACT

	NORMAL BOWEL EVACUATIONS	DIARRHEAL BOWEL MOVEMENTS FOLLOWING EPSOM SALTS	TOTAL
Patients	996	127	1,123
Examinations positive for cells	7	4	11
Examinations negative for cells	1,953	194	2,147
Total examinations	1,960	198	2,158

Out of a total of 2,158 examinations, only 11 were positive for cells. The positive examinations were of specimens obtained from two patients, 57 and 62 years of age. Although an attempt was made to study these two patients further, it has been impossible to get in touch with them.

When 2,147 out of 2,158 specimens were negative for cells, the results would indicate that cells do not occur in the bowel discharges of normal individuals, even though Epsom salts are given in order to produce a diarrheal movement.

AUTOPSY EXAMINATIONS

With a view to testing the validity of the conclusions drawn from the studies with the so-called normal individuals, which showed that cells were not present in the bowel discharges of normal individuals, specimens of the bowel contents, as well as scrapings from the bowel wall at various locations, were taken during autopsies performed upon seven individuals. The purpose of this procedure was to discover whether the cells that appeared in the bowel contents were exactly similar to those of the scrapings so that the inference could be drawn, with a reasonable degree of confidence, that the presence of cells was due to post-mortem degeneration.

From this study it was discovered that the cells found in the bowel contents were exactly the same as the cells found in the scrapings taken from the same areas. Thus it can be affirmed that the cells were the result of post-mortem degeneration and that they were not present before the post-mortem degenerative processes began. These findings are in complete agreement with those of Schmidt and Strausburger,[12] given in their classic publication of 1910.

TABLE II

AUTOPSY FINDINGS IN 7 PATIENTS

LOCATION	BOWEL CONTENTS			SCRAPINGS OF BOWEL WALL		
	TOTAL SPECIMENS	CONTENTS		TOTAL SPECIMENS	SCRAPINGS	
		POSITIVE	NEGATIVE		POSITIVE	NEGATIVE
Stomach	4	4	0	4	4	0
Duodenum	4	4	0	4	4	0
Jejunum	4	4	0	4	4	0
Terminal ileum	7	4	3	7	7	0
Cecum	6	4	2	6	6	0
Hepatic flexure	7	3	4	7	7	0
Transverse colon	6	4	2	5	5	0
Splenic flexure	6	4	2	6	6	0
Sigmoid	7	5	2	7	7	0

Moreover, the cells found in the scrapings from various levels of the bowel are entirely different from the cells found under pathologic conditions in the bowel discharges of living persons. The four chief types of cell found in the scrapings were (1) large wedge-shaped cells with granular cytoplasm and large oval nuclei composed of heavy rings and containing granules; (2) tall, columnar cells with a finely granular cytoplasm, the nuclei of which were large and oval-shaped with heavy rings and containing granules; (3) cells, irregular in shape, with a granular cytoplasm; their nuclei were oval to round but had very delicate rings which constituted the nuclear membrane; granules were included within these nuclei also; and (4) square cells with granular cytoplasm and small, round, solid nuclei. Polymorphonuclear leucocytes, small round cells, or macrophage cells were not at any time observed in the scrapings. The findings were the same for specimens in the wet methylene blue cover slip preparation as for those which were stained with iron hematoxylin in order to obtain permanent mounts.

DOG AUTOPSIES

Because patients had died of pathologic conditions that might have rendered the bowel tract abnormal, and also because in each instance several hours had elapsed between the time of death and the performance of the autopsy examination, the comparative study of bowel contents and scrapings was necessary. But in order to clarify the problem still further, three apparently normal dogs were given lethal doses of sodium amytal intravenously. As soon as respiration had ceased and before the blood had begun to coagulate, their abdomens were opened. Specimens of bowel contents and scrapings of the mucosa were taken from three levels of the colon and also from the terminal ileum. In each instance the specimens of bowel contents were entirely negative for cells, while scrapings of the mucosa showed cells characteristic of each of the levels of the colon and the terminal ileum from which the specimens were taken.

SUMMARY AND CONCLUSIONS

Problems connected with the diagnosis of pathologic change in the bowel mucosa would be simplified considerably if accurate information were obtained by microscopic examination of the bowel discharges. In order to determine whether or not cellular exudates should be expected in normal bowel discharges,

control observations were made in 1,123 patients who stated that they had no complaints referable to the gastrointestinal tract. A total of 2,158 specimens were examined from these so-called normal patients and, with the exception of 11, they were negative for cells. The eleven positive specimens came from two patients, aged 57 and 62 years, who could not be traced for subsequent study. One hundred ninety-eight specimens were diarrheal movements following dosage with Epsom salts.

Bowel contents from several different levels in the gastrointestinal tract were taken during the autopsy examinations on seven individuals. Scrapings were obtained from the bowel walls at the same locations. The cells found in the bowel contents were exactly the same as those of the scrapings from the same location, while there was also evidence to prove that the cells in the bowel contents were the results of post-mortem degeneration.

Three dogs were given lethal doses of sodium amytal and their abdomens were opened as soon as respiration had ceased. Specimens of the bowel contents from three locations in the colon and from the terminal ileum were entirely negative for cells.

From the three sets of observations, the conclusion may be drawn that if no pathologic condition is present, cells will not be found when microscopic examination is made of the bowel discharges.

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ELECTROCARDIOGRAPHIC CHANGES INDUCED BY EXERCISE IN THE DIAGNOSIS OF CORONARY INSUFFICIENCY

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THAT spontaneous attacks of angina pectoris will occasionally produce changes in the electrocardiogram resembling coronary thrombosis was shown by Feil and Siegel[1, 2] and Parkinson and Bedford.[3] The mechanism of such changes is believed to be the same in both cases, namely, local asphyxia and anoxemia of the heart muscle due to acute interference with the blood supply in a localized area of the heart, temporary in the first case and permanent in the second.

If such is the case, any method employed to bring about rapid local asphyxia or anoxemia of the heart muscle should produce electrocardiographic changes. Under equal adverse conditions local asphyxia or anoxemia should develop much faster and to a greater degree in the presence of coronary insufficiency than in the normal state. Electrocardiographic changes, therefore, developing under such conditions, should indicate the presence of coronary insufficiency.

The methods employed to bring about the acute local asphyxia and anoxemia of the heart were continuous rebreathing of the same air, used by Rothchild and Kissin,[4] and by Katz, Hamburger, and Schultz[5]; the use of epinephrine by Levine, Ernstein, and Jacobson,[6] and Katz, Hamburger and Lev[7]; breathing of air consisting of 10 per cent oxygen and 90 per cent nitrogen by Levy, Bruenn, and Russell[8]; and the exercise test by Wood, Wolferth, and Livezey,[9] Whitten and Herndon[10] and, to a limited extent, by Bierring, Larsen, and Nielsen.[11]

Of these methods, the first two were found to be unreliable and dangerous by Katz, Hamburger, and Schultz,[5] and by Katz, Hamburger, and Lev.[7] The sponsors for the exercise test claim to have observed definite electrocardiographic changes after exercise in some patients with angina pectoris which were not present in normal persons. Thus Wood, Wolferth, and Livezey found that normal individuals showed no important changes in the QRS complex. There was merely an occasional deviation of the electrical axis to the right. The T wave also showed no change from a positive to a negative phase. A negative T wave changed occasionally to positive or a positive wave became more prominent. In half of their anginal patients, on the other hand, they noted a depression in the S-T segment and changes in the T wave from positive to negative. Whitten[12] also found frequent changes in the T wave in the anginal cases after exercise, but added that "further observations will probably reveal that one or more of these changes produced in the angina cases by exercise may occur in other than anginal cases." Bierring, Larsen, and Nielsen observed electrocardiographic changes after exercise in four anginal persons and stated that a few controls did not show any changes.

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The object of the investigation covered in this paper was to determine if exercise actually produced different electrocardiographic changes in an abnormal rather than a normal heart. The test was carried out on 112 persons. Fifty-four had normal hearts; 26 had hypertension, 9 had questionable coronary changes, and 23 had unmistakable evidence of coronary artery disease.

In each case a tracing was obtained in the three conventional leads just before exercise, soon after exercise, two minutes later, and in some cases, again in four to six minutes. Because electrocardiographic changes may occur on mere change in posture, as shown in a previous communication,[13] all tracings both before and after exercise, were obtained in the standing position.

In the noncardiac group there were cases of psychoneurosis, bronchial asthma, obesity, diabetes, and the menopausal syndrome. Thirty-five persons, 10 of whom were hospital interns, were perfectly normal individuals with no complaints. Of the 23 persons with definite evidence of coronary disease, 10 had effort angina, 1 had recurring attacks of typical angina occurring spontaneously, and the rest had such symptoms as effort dyspnea, weakness, gastric disturbances, or precordial discomfort coming on after exertion. In the hypertensive group, the usual symptoms were palpitation, weakness, occasional dyspnea, and precordial pain. Some persons were symptom free.

It will be observed that in our study we took persons with coronary disease as a group, and did not limit it merely to those patients with the anginal syndrome as previous authors have done. The reason for this is that we were interested in learning whether coronary insufficiency as such, regardless of what its clinical manifestations might be, will produce different electrocardiographic changes following exercise than otherwise normal hearts. This applies also to the hypertensive group where at least relative coronary insufficiency is assumed to exist in many cases.

ANALYSIS OF THE ELECTROCARDIOGRAMS

P-Wave Changes.—Only 27 persons showed changes in the P wave soon after exercise. Of these, 19 were normal, 5 coronary, and 3 hypertensive. The changes consisted of increase in its height from 0.5 to 1 mm. and change from a rounded to a more pointed form. In some the changes were still present at the end of four to six minutes. In others the voltage became lower than that of the pre-exercise level, by that time.

Changes in the QRS complex were observed frequently and consisted of diminution or increase in voltage and slight shift in the axis to the right or left. The incidence of changes in voltage is shown in Table I. Such changes occurred more often in the normal than in the coronary, questionable coronary, and hypertensive groups. No prediction could be made as to the type of change in the various groups.

Immediately after exercise more persons showed diminished rather than increased voltage. The reverse was true two to six minutes later. The maximum variation in voltage of the QRS complex in the lead showing the greatest change was 6 mm., the usual being 2 to 3 mm. The greatest fluctuations occurred in the normal group.

TABLE I

Number and percentage of persons in each group, showing no change, diminution, or increase in heights of the R and T waves in the three leads at various periods after exercise. The findings before exercise were used for comparison. All persons had records soon after exercise, but no records were obtained of some persons two to six minutes after exercise.

NO. OF CASES	DIAGNOSIS	ELECTROCARDIOGRAM OBTAINED	R I						R II						R III					
			NO CHANGE		DIMINISHED		INCREASED		NO CHANGE		DIMINISHED		INCREASED		NO CHANGE		DIMINISHED		INCREASED	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
54	Normal heart	Soon after exercise	8	14.9	40	74.0	6	11.1	14	26.1	31	57.3	9	16.6	10	18.5	23	42.6	21	38.9
		Two to six minutes later	19	35.3	13	24.0	22	40.7	17	34.2	12	20.3	25	45.5	14	26.0	18	33.3	22	40.7
26	Hypertension	Soon after exercise	4	15.3	20	77.0	2	7.7	9	34.7	13	50.0	4	15.3	8	30.7	10	38.6	8	30.7
		Two to six minutes later	10	38.6	6	22.8	10	38.6	11	42.3	6	23.0	9	34.7	13	50.1	8	30.7	5	19.2
23	Coronary sclerosis	Soon after exercise	10	43.4	12	52.3	1	4.3	8	34.9	10	43.4	5	21.7	10	43.4	7	30.5	6	26.1
		Two to six minutes later	9	39.0	5	22.0	9	39.0	10	43.4	7	30.5	6	26.1	9	39.0	8	34.9	6	26.1
9	Questionable coronary	Soon after exercise	1	11.2	8	88.8	0		3	33.3	5	55.5	1	11.2	5	55.5	3	33.3	1	11.2
		Two to six minutes later	5	55.4	2	22.3	2	22.3	4	44.4	2	22.3	3	33.3	5	55.4	2	22.3	2	22.3

NO. OF CASES	DIAGNOSIS	ELECTROCARDIOGRAM OBTAINED	T I						T II						T III					
			NO CHANGE		DIMINISHED		INCREASED		NO CHANGE		DIMINISHED		INCREASED		NO CHANGE		DIMINISHED		INCREASED	
			No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
54	Normal heart	Soon after exercise	14	22.3	20	37.0	22	40.7	10	19.5	23	42.6	21	38.9	12	22.2	21	38.9	21	38.9
		Two to six minutes later	28	55.7	13	24.0	11	20.3	26	48.1	14	25.9	14	25.9	17	31.5	19	35.2	18	33.3
26	Hypertension	Soon after exercise	8	30.7	7	27.0	11	42.3	5	19.2	5	19.2	16	61.6	7	27.0	6	23.0	13	50.0
		Two to six minutes later	13	50.0	5	19.3	8	30.7	9	34.7	6	23.0	11	42.3	13	50.0	7	27.0	6	23.0
23	Coronary sclerosis	Soon after exercise	4	17.3	8	34.9	11	49.8	4	17.9	5	21.7	14	60.4	9	39.0	6	26.1	8	34.9
		Two to six minutes later	9	39.0	7	30.5	7	30.5	12	52.2	7	30.5	4	17.3	12	51.9	6	26.1	5	22.0
9	Questionable coronary	Soon after exercise	2	22.3	4	44.4	3	33.3	4	44.4	2	22.3	3	33.3	3	33.3	4	44.4	2	22.3
		Two to six minutes later	3	33.3	5	55.5	1	11.2	3	33.3	4	44.4	2	22.3	3	33.3	6	66.7	0	

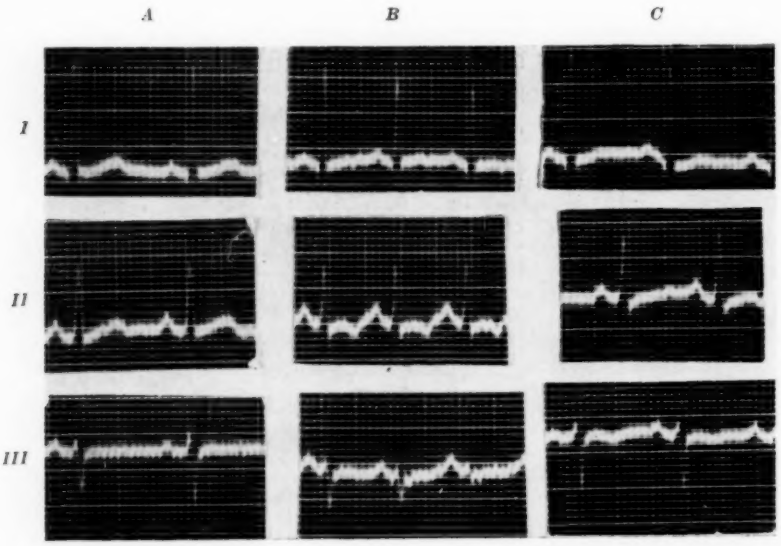

Fig. 1.—From a male 33 years old, with questionable coronary sclerosis. *A*, before, *B*, soon after, and *C*, three minutes after exercise. T-wave changes are noted in the first and second leads soon after exercise which did not return to the pre-exercise level in three minutes.

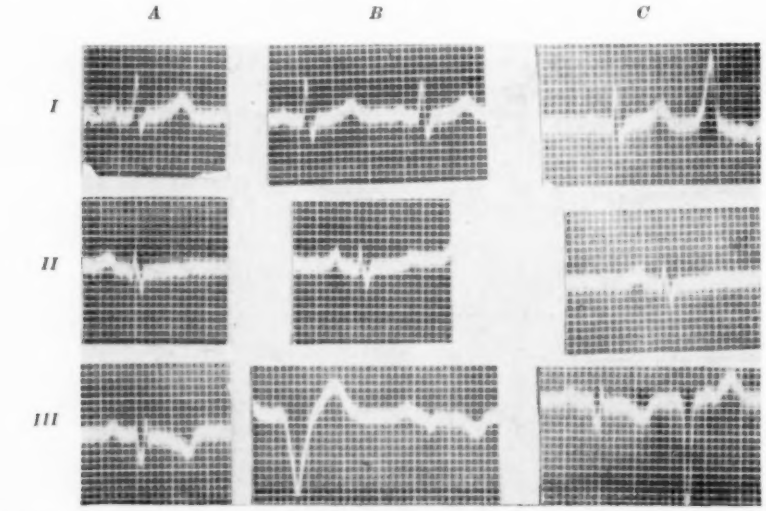

Fig. 2.—From a male 54 years old, with definite coronary disease and an attack of coronary occlusion three months before. *A*, before, *B*, soon after, and *C*, four minutes after exercise only trivial changes are noted in the T wave. QRS voltage in the third lead diminished and ventricular premature contraction appeared after exercise.

Changes in the R-T or S-T segment occurred in 26 persons. The changes consisted of rounding, elevation, or depression. Of these, 6 normal persons, 3 with coronary disease, and 2 with hypertension had normal segments in one, two, or all three leads before exercise which became depressed or elevated soon after exercise, and in some cases remained so for two to six minutes. The greatest elevation or depression in any case was never more than 1 mm. Two normal persons had very slight depression and rounding before exercise, which returned to the isoelectric level afterward. One normal person had some depres-

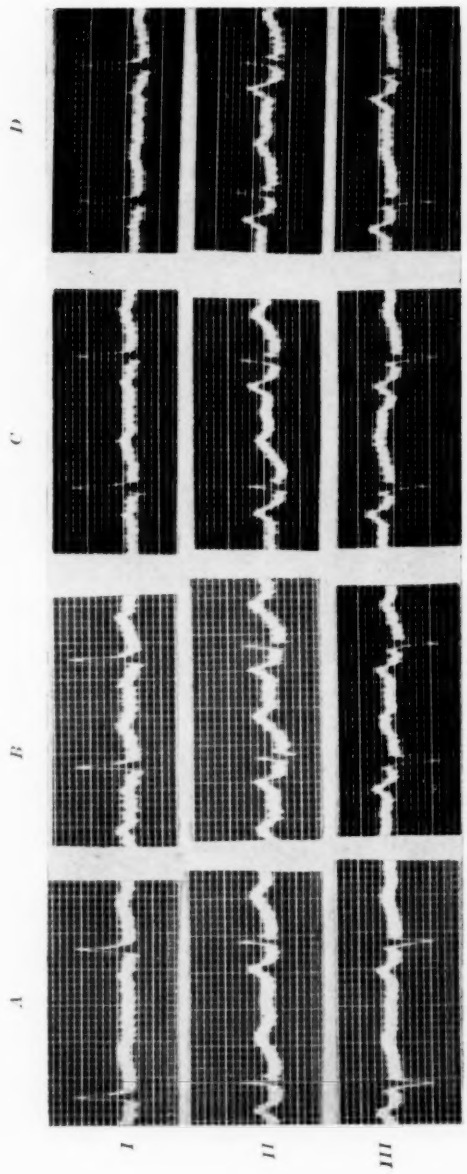

Fig. 3.—From a male 60 years old, with coronary sclerosis. *A*, before, *B*, soon after, *C*, two minutes after, and *D*, six minutes after exercise. The T wave is increased in voltage and is more pointed soon after exercise, remaining so two minutes after exercise. The electrical systole is shortened.

sion and another some elevation before exercise, which returned to the isoelectric level later. In one person with coronary disease, a pre-existing depression of the R-T segment in the second lead became more depressed soon after exercise and returned to the original condition two to six minutes later. There were 5 persons with coronary disease with slight depression of the R-T segment, one with elevation and 2 with rounding before exercise, which remained the same afterward.

Changes in the T wave occurred frequently and consisted of increase or decrease in voltage, more rapid conduction time, and slight change in appear-

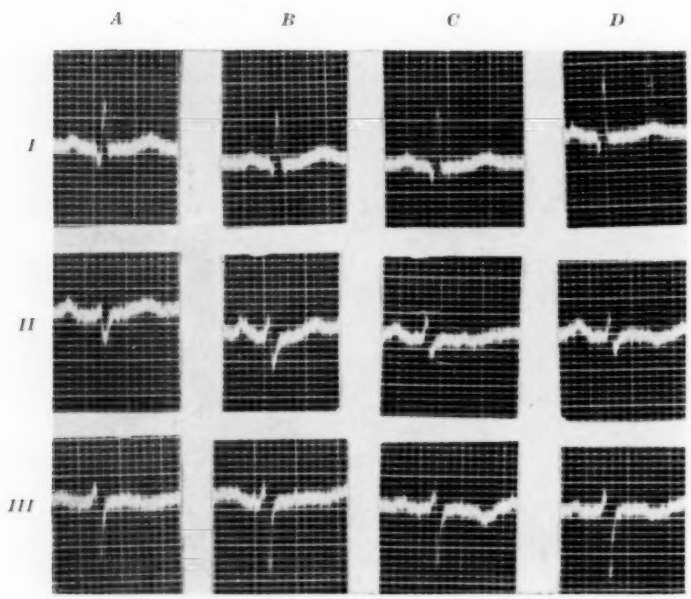

Fig. 4.—From a female, 48 years old, with moderate hypertension. *A*, before, *B*, soon after, *C*, two minutes after, and *D*, five minutes after exercise. There are changes from positive to negative T wave in second lead, and from mildly negative to greater negative T wave in third lead two minutes after exercise, remaining the same five minutes later. Slight change in QRS voltage is also noted.

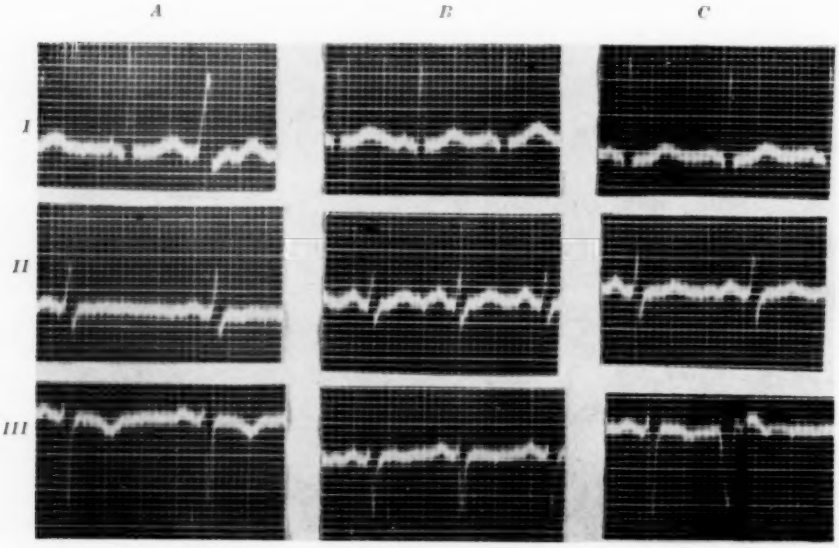

Fig. 5.—From a male 49 years old, with hypertension. *A*, before, *B*, soon after, *C*, two minutes after exercise. Voltage of QRS complex diminished and of T wave increased soon after exercise. The QRS returned to pre-exercise level in two minutes, but the T wave still remained somewhat higher at that time. A ventricular premature contraction is seen before and two minutes after exercise.

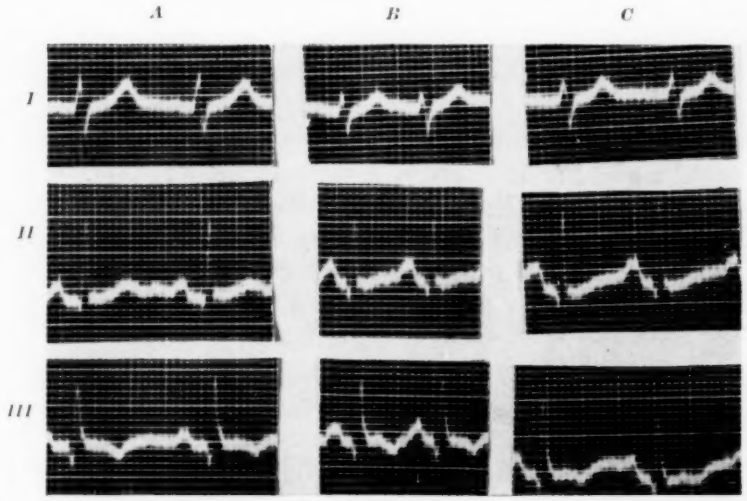

Fig. 6.—From a male 36 years old, with no clinical evidence of heart disease. *A*, before, *B*, soon after, *C*, two minutes after exercise. Voltage of QRS complex and T waves is diminished and there is some rotation of the axis to the right soon after exercise, remaining approximately the same two minutes after exercise.

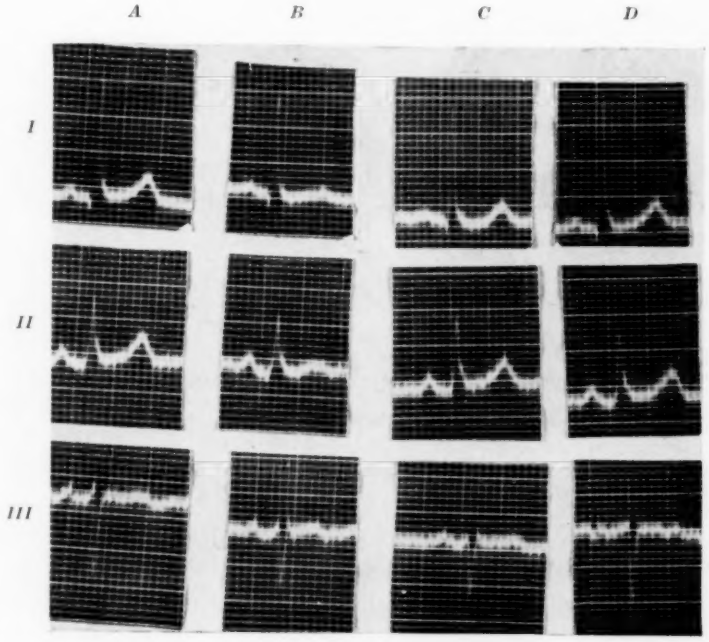

Fig. 7.—From a male 64 years old, presenting evidence of coronary and aortic sclerosis with the anginal syndrome. *A*, before, *B*, soon after, *C*, two minutes after, *D*, five minutes after exercise. Voltage of QRS and T is diminished soon after exercise and returns to pre-exercise level in two minutes, at which time the S wave in the third lead still remains of low voltage. In five minutes complexes are exactly the same as those in *A*.

ance from a rounded to a pointed form. As shown in Table I, the changes were observed more often in the first and second leads in the coronary group and in the third lead in the normal group. The maximum change in voltage was 1.5 mm., the usual being less than 1 mm.

In the normal group the T wave in the first lead never showed any changes from positive to isoelectric or negative. One person, however, showed such change in the second lead (Fig. 6) and six persons in the third lead. In six other persons an isoelectric T wave in the third lead changed to positive, and in nine persons negative T wave changed to isoelectric soon after exercise but returned to the original condition two to six minutes later. In five persons a positive T wave in the third lead before exercise remained so soon after, but was changed to negative or diphasic two to six minutes later.

There was one male, 33 years old, who complained of left precordial pain. Because his first heart sound was diminished in intensity and there was some peripheral arteriosclerosis, he was put in the group of questionable coronary disease. His electrocardiogram before exercise (Fig. 1) showed merely left axis deviation. Soon after exercise the T wave became almost isoelectric in the first lead and negative in the second lead, remaining so at the end of three minutes.

TABLE II

Persons in the various groups, showing range of heart rate, percentage increase in rate after exercise, percentage of persons returning in the pre-exercise level, and maximum percentage increase still present among those who did not return to pre-exercise level. The heart rates were calculated in all persons from the electrocardiograms obtained in the standing posture.

GROUPS	ORIGINAL HEART RATE	PERCENTAGE INCREASE IN RATE SOON AFTER EXERCISE	PERCENTAGE OF CASES THAT RETURNED TO PRE-EXERCISE LEVEL AT END OF		MAXIMUM INCREASE IN RATE OVER PRE-EXERCISE LEVEL STILL PRESENT AT END OF	
			2 min.	4 to 6 min.	2 min.	4 to 6 min.
Normal	60 to 115	8.8 to 51.2	27.7	63.7	27.0%	25.6%
Hypertension	65 to 120	2.1 to 46.3	30.7	55.7	39.1%	23.0%
Coronary	60 to 125	6.5 to 43.8	34.7	81.3	20.0%	20.0%
Questionable coronary	92 to 115	10.2 to 46.5	44.4	72.9	29.5%	23.0%

In the group with coronary disease, one person (Fig. 7) had positive T waves in the first and second leads, which became almost isoelectric immediately after exercise and returned to the original condition later. In one patient a positive T wave in the first lead before exercise changed to isoelectric soon after exercise and was still in that condition two minutes later. In one person a diphasic T wave in the first lead before exercise changed to mildly positive after exercise and to greater positivity in two minutes. Another person, showing an isoelectric T wave in the first lead before exercise, became positive soon after exercise, and returned to isoelectric again two minutes later. Similar changes were noted in the second lead alone in two persons, and in the second and third leads in two other persons. All others in this group showed about the same T-wave changes as the group with normal hearts.

In the hypertensive group an isoelectric T wave in the second lead changed to positive immediately after exercise (Fig. 5) in two persons and two minutes after exercise in two others. Similar changes were noted in two persons in the

third lead. In one person a positive T wave in the second lead changed to diphasic soon after exercise and again to positive two minutes after. In four persons, the T-wave changes in the third lead were the same as in normal cases.

Effect of Exercise on the Heart Rate.—The acceleration of the heart rate following exercise is shown in Table II. It will be seen that individuals with normal hearts have a tendency for a somewhat greater acceleration soon after exercise, and such acceleration lasted longer than in persons with abnormal hearts. Inasmuch as the electrocardiograms from which the rates were determined were obtained in the standing postures, the rates in general are higher than the usual average.

DISCUSSION

If we consider the electrocardiographic manifestations occurring during the interval between the inception of the Q wave and the end of the T wave as the algebraic summation of spread and recession of the fractional components of the electrical potential in the individual cardiac muscle fibers in the ventricles, changes in the physical state of the heart muscle should theoretically produce changes in the resulting complexes.

Exercise is one condition where the physical state of the heart should undergo rapid change, and such change should occur earlier and be greater in coronary disease than in the normal heart. Anoxemia and the accumulation of waste products should occur much quicker and to a greater extent in coronary insufficiency. Theoretically, therefore, the exercise test should help us in the diagnosis of coronary insufficiency. Practically, however, no marked differences were noted in our findings in persons with normal and abnormal hearts following exercise. Comparatively few cases of coronary disease showed changes after exercise that were not found also in the normal.

That the heart rate, as determined from the electrocardiogram, did not return to the pre-exercise level in some cases as long as six minutes after exercise and that this occurred more frequently in normal hearts than in coronary disease appears to be contrary to belief that the heart rate must come down to the pre-exercise level within two minutes after exercise if we are to consider the heart normal. This might partly be due to the fact that all determinations were done in the standing postures.

In assigning significance to the findings presented in this paper, the amount of exercise employed in each case is probably an essential factor. Individuals who presented symptoms of dyspnea, palpitation, fatigue, precordial oppression or pain early did not do as much exercise as others. This may partly account for a more sustained effect of exercise on the electrocardiogram in the normal heart. If this be so, the subjective reaction of the individual, more than the electrocardiographic findings, should be considered the important criterion of myocardial insufficiency.

SUMMARY

Electrocardiographic studies were carried out in 112 persons before and after exercise. Fifty-four had normal hearts, 26 had hypertension, 23 coronary sclerosis, and 9 questionable coronary disease. Many of the persons showed increased voltage of the P wave, increased or diminished voltage of the QRS

complex, slight elevation or depression of the R-T or S-T segment, and increased or decreased voltage of the T wave. These changes occurred soon after exercise and lasted in some cases as long as six minutes. In two persons with coronary and in one with suspicious coronary disease a positive T wave in the first lead or first and second leads before exercise changed to negative or isoelectric T wave after exercise. In no normal persons did such changes occur in the first and second leads, but several showed it in the third lead. The usual changes were about the same in cases with normal as with abnormal hearts, except that in the normal heart they were somewhat more marked and lasted longer. In the normal heart there was a tendency for a greater increase in the heart rate after exercise.

We may conclude from these findings that the electrocardiogram does not usually show characteristic changes after exercise that could be taken to indicate the presence of coronary insufficiency. In an occasional case it may be of value. The subjective reaction is perhaps still the most important criterion of the cardiac functional state.

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HYPERTENSION AND OBESITY*

A Statistical and Clinical Study of 10,883 Individuals

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TO WHAT extent is obesity related to hypertension? Medical literature does not conclusively answer this question. Although a wide variety of groups has been studied, there has been no standardization of either the hypertension or the obesity concept. The meaning of the term "obesity" is not clear. Surely weight alone does not measure it, and a clinical impression or a "build group," while helpful, is not a final definition of obesity. There is, therefore, need for a more adequate treatment of this subject.

Since the first rough studies of blood pressure were published, the most common associated physical finding has been overweight. Every study of the blood pressure-weight relationship has shown that the average blood pressure of a large group of persons rises with increments of weight. Yet there are conflicting opinions confronting the all-inclusive statement that blood pressure correlates to weight. Bowes[1] in 1917 found no weight relation to blood pressure. However, his group consisted of a small number of aged individuals, most of whom were afflicted with diseases other than hypertension alone. Post and Stieglitz[2] and Schulze and Schwab[3] reported groups of hypertensive persons in whom obesity was relatively rare. Geil and Secher,[4] though over 50 per cent of their hypertensive patients were obese, concluded that "ostensibly obesity has no significance in hypertension." Mosenthal,[5] while recognizing the relation, states that the influence of weight has probably been exaggerated.

The literature on obesity and hypertension is also confusing on the question of what ages are liable to the overweight-hypertension relationship. Symonds,[6] arranging his study by build groups on a weight per unit height basis somewhat similar to ponderal index, found an increase in pressure with an increase in relative obesity for all ages. The Actuarial Society of America,[7] on the other hand, found that two-thirds of all individuals ten pounds or more overweight have an elevated blood pressure, but specified that this relation existed only for ages over forty. Another confusion arises from the work of Huber,[8] who showed that 53 per cent of underweights and 6 per cent of overweights had low blood pressure, while 21 per cent of underweights and 14 per cent of overweights had high blood pressure.

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The source material for this study was gathered from records accumulated during the past ten years by the former Life Extension Institute, Chicago; the West Side Y. W. C. A., Chicago; the Student Health Service of the University of Chicago; and from the files of private practice.

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A survey of the literature on the problem can be broken into a number of subdivisions according to the methods of study. Some authors have used the clinical impression of obesity in its extreme state and have noticed, many times without specifically looking for it, the common association with an elevated blood pressure.[9-13] Conversely, other authors working with hypertensive persons have found a large percentage of their groups to be obese.[14-18]

Fewer data are available in regard to the opposite relationship between underweight and low blood pressure. The conclusions of Huber[8] and Brower[19] point out a very strong tendency for low blood pressure to be associated with underweight.

Another important source of data concerning the weight-blood pressure relationship has been furnished by the insurance studies. However, the insurance medical examiners have never used a standardized technique for recording weights. It has been only during the past few years that insurance examiners have carried scales. Yet even now scales are rarely used, and what is more, weights are never measured in the nude subject; hence a variable weight of clothing is recorded. In the past the insurance doctor merely asked the examinee what his weight was, and when in doubt, used the nearest drugstore scale. Medical directors themselves have recognized the unreliability of these weights, and, accordingly, have used a synthetic weight measure calculated from a formula embodying height, chest, and abdominal measures. It is obvious that such an indirect measure cannot give scientifically accurate results. Similar criticisms have been made on the lack of standardization of the technique of measuring blood pressures for insurance examination. The dissatisfaction of many medical directors with the standardization of their examinations is promising of conclusive work in the future. Bearing these criticisms in mind, the insurance studies have all shown that blood pressure increased with overweight and decreased with underweight.[6, 20-24]

A further criticism of much of the literature on weight and blood pressure is that there have been few studies that have established an index of obesity as differentiated from pure weight alone. Most of the work that has recognized the difference between weight and obesity has circumvented the problem by creating "build groups," accurate enough to differentiate gross extremes of obesity but not satisfactory in delineating the accumulation of adiposity within widely varying types of build.[24-26]

In order to overcome these criticisms and to determine more accurately the relationships inherent in the correlation of overweight and hypertension, we abstracted the periodic physical examinations of 10,883 individuals, apparently healthy or with minor complaints.* In each case the examination was made under standardized conditions by one of six physicians. Identical methods were used in measuring the blood pressure, height, weight, chest, and abdomen, and an effort was made to control the conditions of examination as far as was possible. All physical measures were taken on the nude subject. Weight was measured on a beam scale made by the Continental Company, and recorded to the nearest pound for the purpose of this study. Height was measured on a specially designed apparatus wherein the heels of the subject were placed against the backboard of a platform and the shoulders thrown back against

*For a complete analysis of the blood pressure distribution in these groups see Robinson, S. C., and Brucer, M.: The Range of Normal Blood Pressure, Arch. Int. Med., September, 1939.

a rod; the head was tilted to obtain the maximum height to one-fourth of an inch. The chest measure was taken with a steel tape. In the male it was taken about an inch above the nipple line during quiet breathing; in the female the chest measure was taken above the breast tissue well above the nipple line. The abdominal measure was at the level of the umbilicus. Blood pressure measurement was made with mercury manometers on the left arm of the sitting subject. The auscultatory technique and the fourth diastolic phase were used.

For the study of weight and blood pressure, 7,478 male and 3,405 female records were examined. The physical measures herein correlated were distributed in a manner to be discussed next.

PONDERAL INDEX AND BLOOD PRESSURE

Despite the obvious correlation of weight to blood pressure, conclusions drawn from the weight measure alone are highly inaccurate. Except at the extreme ends of an array, weight alone does not show a necessary correlation of obesity to blood pressure. For example, of two men—the one very short, the other very tall—each weighing 150 pounds, the short man would be overweight while the tall one would be underweight. This simple example makes obvious the necessity for rejecting weight as a single criterion of obesity. In order to relate obesity to hypertension it is first necessary to determine the degree of obesity within specific height groups. The clinical impression of obesity, as used by some authors, has palpable shortcomings, and likewise, the typing of individuals by build groups, as is practiced by insurance actuaries, is not entirely without fault, for this assignment of individuals into groups bears neither the statistical convenience nor the accuracy of a calculated index of relative obesity. Perhaps the simplest and most easily available method of ranging individuals relative to degree of obesity is with the use of the ponderal index. The ponderal index is defined as the simple ratio of weight in pounds divided by height in inches.* Thus of two men, each weighing 150 pounds, the one 74 inches tall would have a ratio of 2.0, a lightweight index number, while the short one at 60 inches would have a ratio of 2.5, a heavyweight index number. It is easily seen why such an index is far more accurate and practical than weight alone, because it provides a single statistical unit combining height and weight, thus giving us a better numerical picture of obesity.†

*Ponderal index has also been defined in terms of kilogram-centimeter ratio by some, chiefly European anthropometricians. The pound-inch unit, however, corresponds more closely to the American "build group" determinations and is in more common usage. The height distribution of the 7,478 males and 3,405 females used in this study is very much like the distribution to be expected from studies of large samples of the United States population. The mean height of the males was 68.4 inches; the mode was 68.1 inches. A standard deviation of 2.8 showed the males to concentrate within the height range from 65.6 to 71.2. The mean female height was 63.6 and the mode 63.6, with a standard deviation of 2.5, showing the female height to concentrate in the range from 61.6 to 66.1.

†Because height is unidimensional while weight is tridimensional, it has been suggested that the cube of height or the cube root of weight be used in calculating the index. Height squared has also been used. While these factors may correct the small error present in the extremes of the ponderal index distribution, this error is smaller than the class interval used in grouping our dispersion. The coefficients of correlation of these various modifications of the weight-height index are as follows:

	Correlation	7,478 *Men*	3,405 *Women*
W/H	to systolic pressure =	0.196 ∓ 0.011	and 0.262 ∓ 0.022
W/H^2	to systolic pressure =	0.183 ∓ 0.022	and 0.256 ∓ 0.020
W/H^3	to systolic pressure =	0.198 ∓ 0.022	and 0.275 ∓ 0.022
$2\sqrt{W}/H$	to systolic pressure =	0.171 ∓ 0.023	and 0.283 ∓ 0.022
$3\sqrt{W}/H$	to systolic pressure =	0.190 ∓ 0.022	and 0.286 ∓ 0.022

Using the formula $\sigma D = \sqrt{\sigma^2\theta_1 + \sigma^2\theta_2 - 2r_{12}\sigma\theta_1\sigma\theta_2}$, the difference between any two of these correlations would have to be at least 0.062 in order to be significant. The actual difference in no

THE PONDERAL INDEX DISTRIBUTION

The distribution of ponderal index among 7,478 males and 2,325 females shows that the ratio ranges from 1.5 in extremely lightweight persons to over 3.7 in extremely heavyweight persons. The mean male ponderal index was 2.3, with a slightly lower mode at 2.2. A standard deviation of 0.3 showed that the bulk of the males was grouped in the range from 2.0 to 2.6. The female distribution showed a mean ponderal index slightly lower than that of males, at 2.2, with a mode at 2.0; the standard deviation was 0.4, which indicates that the bulk of the females was in the range from 1.8 to 2.6.

By clinical impression and comparison with standard tables it was decided to divide the group tentatively into lightweights (a ponderal index up to 2.0), mediumweights (a ponderal index from 2.0 to 2.4), and heavyweights (a ponderal index of 2.5 and over). These groups merge one into the other; in the original statistical analysis much finer subdivisions were used. This division classed 17 per cent of the males and 38 per cent of the females as lightweight,* 57 per cent males and 41 per cent females as mediumweight, and 26 per cent males and 21 per cent females as heavyweight.

TABLE I*

THE RELATIONSHIP OF AGE TO PONDERAL INDEX

AGE GROUPS	UNDER 20	20-29	30-39	40-49	50-59	60 AND OVER	ALL AGES
7,478 Males							
No. of cases	113	1652	2387	1967	943	416	7478
Mean ponderal index	1.96	2.31	2.32	2.37	2.42	2.22	2.30
Mode ponderal index	2.04	2.06	2.25	2.45	2.45	2.25	2.25
Per cent lightweight	51	26	16	12	11	12	17
Per cent heavyweight	6	13	25	35	36	30	27
2,325 Females							
No. of cases	46	595	797	563	232	92	2325
Mean ponderal index	1.95	1.98	2.16	2.28	2.48	2.28	2.10
Mode ponderal index	1.85	1.85	2.05	2.25	2.36	2.17	1.95
Per cent lightweight	72	63	38	20	21	21	38
Per cent heavyweight	6	7	18	33	40	27	21

*All tables in this paper are condensed from detailed correlation tables. The statistical measures have been expressed to the nearest unit.

With an increase in age the ponderal index increases for both male and female groups (Table I). The twenty-year-old males have a mean ponderal index of 1.96, which steadily rises as the age increases until the peak ponderal index of 2.42 is reached toward the end of the fifth decade. Note that while only 6 per cent of the very young men are heavyweight, fully 35 per cent of the men in the age groups 40 to 60 are heavyweight. The female group shows a similar tendency. Starting at an index of 1.95, comparatively the same as

Continued from page 809.

instance exceeds 0.027 in the men and 0.030 in the women. Therefore, for the purposes of this study, the complications inherent in the modifications of the weight-height index can add very little to the value of the index. The weight-height index is used in this paper because it is the simplest and easiest to understand.

Some authors use the sitting height instead of the standing height measure. However, the sitting height measurement is not generally taken by the physician; it is unfamiliar and demands more rigid standardization. At any rate, the statistical difference between the two measures is said to be slight.

*The fact that the females were a slightly younger group would modify the difference somewhat.

that of males, the female group gains weight more slowly to reach a peak ponderal index in the sixth decade. While only 6 per cent of the young females are heavyweight, in the 50 to 60 year age group 40 per cent of all females are in the heavyweight class. This increase should *not* be interpreted as a physiologic accretion of weight beyond the third decade. A yearly increase in weight does not take place in the physically active farmer group where obesity is relatively rare,[28] nor is it the rule among primitive peoples[29] where

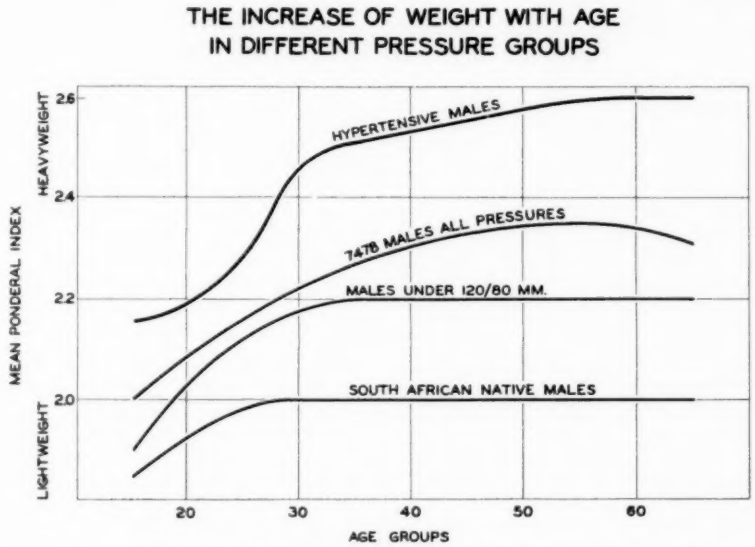

Fig. 1.—In a group of physically active South African natives (30) the maximum weight was reached before the thirtieth year and remained constant throughout adult life. In a civilized group of males, those with low blood pressure followed a similar weight curve, while those with high pressure continued to gain weight over the entire life span.

there is no opportunity for sedentary living. For example, in a study of South African natives, Brodie[30] observed an increase in weight up to age 25, after which the average weight remained at the 135 pound level. These natives were physically active. Only among a sedentary group is there an interference with the normal physiologic processes regulating body weight (Fig. 1). By this interference with the regulation of body weight, the span of life is shortened. The effects of a yearly increase in weight are lucidly illustrated by recent mortality studies.[31, 32] With every increase in weight, beginning with below average levels, there is a progressive increase in gross mortality. That this overweight mortality soon reaches alarming proportions has long been known.[33-35] The high mortality of the obese explains the decrease in mean ponderal index after the fiftieth year, and thus we may surmise that relatively few heavyweights live long enough to be included in the older age groups. Although the peak of overweight in the female occurs about 10 years later than in the male, overweight in the female is accompanied by the same relative mortality tendencies.[36]

THE RELATION OF PONDERAL INDEX TO SYSTOLIC BLOOD PRESSURE

With an increase in relative obesity, as measured by ponderal index, there was a decided steplike increase in systolic blood pressure. The modal pressure

TABLE II

THE RELATION OF PONDERAL INDEX TO BLOOD PRESSURE

	PONDERAL INDEX	LIGHTWEIGHT		MEDIUMWEIGHT		HEAVYWEIGHT			ALL WEIGHTS	CORRELATION DATA
		UNDER 1.70	1.70-1.99	2.00-2.29	2.30-2.49	2.50-2.69	2.70-2.99	3.00 & OVER		
7,478 MALES SYSTOLIC PRESSURE	Under 110 mm.	39	369	644	297	183	85	20	1,637	
	110-119 mm.	20	363	845	478	282	160	52	2,200	
	120-139 mm.	26	384	974	585	497	299	70	2,835	
	140 mm. and over	6	66	218	185	140	131	60	806	
	Total	91	1,182	2,681	1,545	1,102	675	202	7,478	
	Mean pressure	116	117	119	122	124	127	130	121	
	Mode pressure	106	115	117	115	119	124	117	116	
	Per cent under 110 mm.	43	31	24	19	17	13	10	22	
	Per cent under 120 mm.	65	62	56	50	42	36	36	51	
	Per cent 140 mm. and over	7	6	8	12	13	19	30	11	
2,325 FEMALES SYSTOLIC PRESSURE	Under 110 mm.	75	329	257	83	61	39	24	868	
	110-119 mm.	46	229	172	67	49	37	17	617	
	120-139 mm.	27	151	186	73	63	63	28	591	
	140 mm. and over	5	28	56	49	42	43	26	249	
	Total	153	737	671	272	215	182	95	2,325	
	Mean pressure	112	112	117	122	124	127	127	113	
	Mode pressure	107	113	112	115	115	122	124	113	
	Per cent under 110 mm.	49	45	38	31	28	21	25	37	
	Per cent under 120 mm.	79	76	64	55	51	42	43	64	
	Per cent 140 mm. and over	3	4	8	18	20	24	27	11	
7,478 MALES DIASTOLIC PRESSURE	Under 60 mm.	15	109	150	52	17	11	2	356	
	60-79 mm.	57	887	1,962	1,024	644	335	91	5,000	
	80-89 mm.	14	153	453	349	327	221	71	1,588	
	90 mm. and over	5	33	116	120	114	108	38	534	
	Total	91	1,182	2,681	1,545	1,102	675	202	7,478	
	Mean pressure	69	71	73	75	77	80	81	74	
	Mode pressure	67	72	72	72	76	77	82	73	
	Per cent under 60 mm.	16	9	6	3	2	2	1	5	
	Per cent under 80 mm.	79	84	79	70	60	51	46	72	
	Per cent 90 mm. and over	5	3	4	8	10	16	19	7	
2,325 FEMALES DIASTOLIC PRESSURE	Under 60 mm.	30	119	78	29	12	17	9	294	
	60-79 mm.	106	528	439	168	126	95	43	1,505	
	80-89 mm.	14	72	121	50	50	53	24	384	
	90 mm. and over	3	18	33	25	27	17	19	142	
	Total	153	737	671	272	215	182	95	2,325	
	Mean pressure	68	67	72	74	76	76	78	72	
	Mode pressure	62	67	72	72	72	73	72	72	
	Per cent under 60 mm.	20	16	12	11	6	9	10	13	
	Per cent under 80 mm.	89	88	77	72	64	62	55	77	
	Per cent 90 mm. and over	2	2	5	9	13	9	20	6	

CORRELATION DATA	PONDERAL INDEX	SYSTOLIC PRESSURE
Mean	2.31 ± 0.004	121.0 ± 0.20
Std. Dev.	0.328	17.3
Skewness	0.27	0.29
Coef. Var.	14.20	14.34
Coef. Corr.	r = 0.19 ± 0.01	
Corr. Ratio	$\eta = 0.21$	
Test Lin.	$\zeta = 0.006 \pm 0.002$	

CORRELATION DATA	PONDERAL INDEX	SYSTOLIC PRESSURE
Mean	2.19 ± 0.008	117.0 ± 0.41
Std. Dev.	0.402	19.87
Skewness	0.597	0.48
Coef. Var.	18.36	16.98
Coef. Corr.	r = 0.260 ± 0.019	
Corr. Ratio	$\eta = 0.281$	
Test Lin.	$\zeta = 0.021 \pm 0.006$	

CORRELATION DATA	PONDERAL INDEX	DIASTOLIC PRESSURE
Mean	2.31 ± 0.004	74.4 ± 0.12
Std. Dev.	0.328	10.49
Skewness	0.27	0.31
Coef. Var.	14.20	14.10
Coef. Corr.	r = 0.274 ± 0.011	
Corr. Ratio	$\eta = 0.285$	
Test Lin.	$\zeta = 0.006 \pm 0.002$	

CORRELATION DATA	PONDERAL INDEX	DIASTOLIC PRESSURE
Mean	2.19 ± 0.008	71.5 ± 0.24
Std. Dev.	0.402	11.36
Skewness	0.597	0.11
Coef. Var.	18.36	15.89
Coef. Corr.	r = 0.249 ± 0.019	
Corr. Ratio	$\eta = 0.289$	
Test Lin.	$\zeta = 0.022 \pm 0.006$	

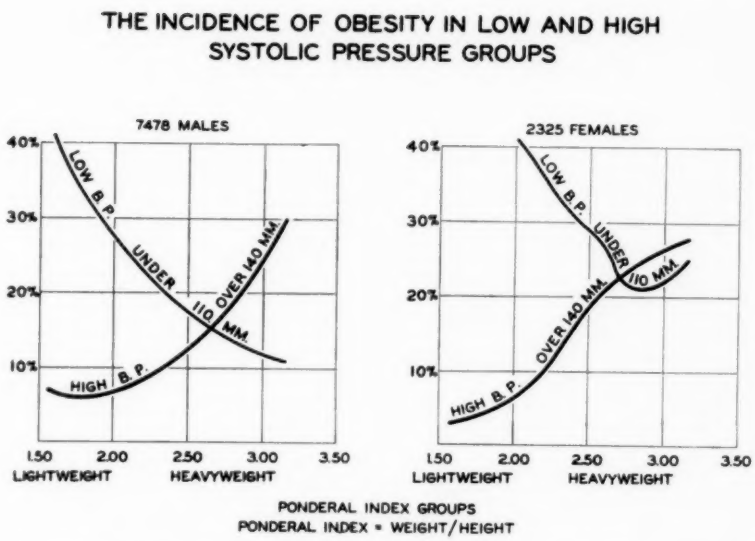

Fig. 2.—The incidence of high pressure is less frequent among the lightweights and increases in frequency as weight increases. On the other hand, low blood pressure is most frequent among the lightweights and sharply decreases with increase in weight.

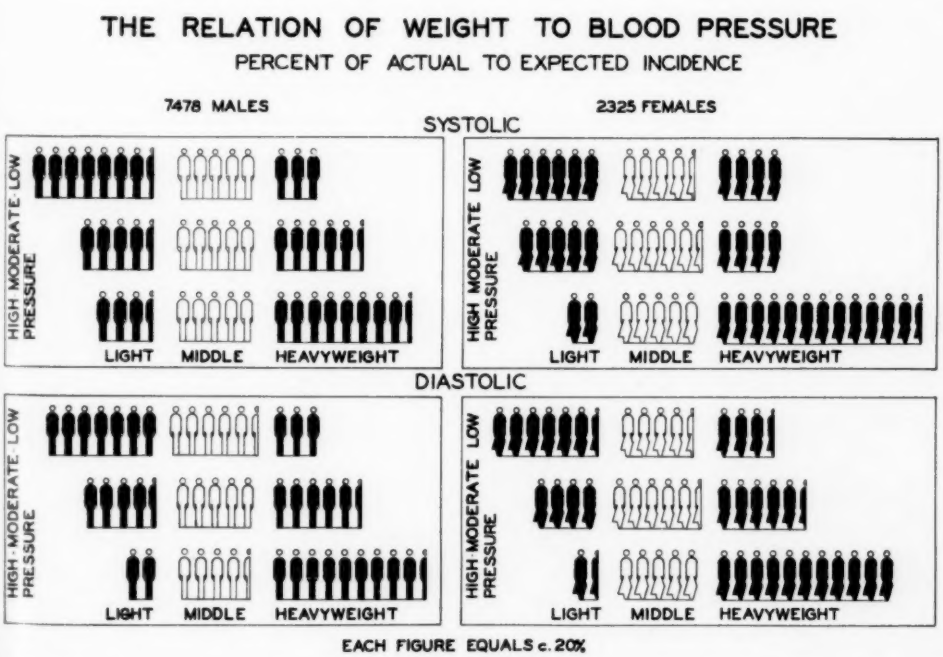

Fig. 3.—High pressures are more common among the heavyweights and low pressures are more common among the lightweights.

increased with an increase in relative obesity, though not as markedly as did the mean.* This indicates that there is not a pound-for-pound, millimeter-for-millimeter rise, but instead, an increasing *incidence* of high blood pressures with each increase in relative obesity (Table II). That this is more striking in the females is to be expected because of the very low pressures we observed in young females at the age when we expect them to have a relatively low weight. Lightweight females have lower pressures while heavyweight females have the same systolic pressures that are observed in men of similar weight.

The relative incidence of low and high pressures in the various weight categories shows this relationship clearly (Fig. 2). Note the high incidence of low pressures (40 per cent) in the lightweight males. This incidence sharply decreases with each increase in weight until among the obese males only one-tenth of the group has low pressures. Conversely, in the incidence of high pressures, the very opposite tendency is apparent. Starting with an incidence of only 7 per cent of high pressures among the lightweight males, there is a slow accumulation of high pressures in the middleweight group and then an accelerated rise to a 30 per cent incidence of high pressures among the obese males.

In the female group the tendency is similar. The lightweight females have a high incidence of low pressures and a low incidence of high pressures, while the heavyweight females have a lower incidence of low pressures (though not as strikingly low as the males) and a higher incidence of high pressures. This crossing of the incidence curves of low and high pressures, as pictured in Fig. 2, is a dramatic illustration of the effects of weight on blood pressure.

TABLE III

PER CENT ACTUAL TO EXPECTED RATIO PONDERAL INDEX TO SYSTOLIC BLOOD PRESSURE

	7,478 MALES		2,325 FEMALES	
	LIGHTWEIGHTS (UNDER 2.0)	HEAVYWEIGHTS (OVER 2.4)	LIGHTWEIGHTS (UNDER 2.0)	HEAVYWEIGHTS (OVER 2.4)
Low pressure (Under 110 mm.)	145	68	135	79
High pressure (140 mm. and over)	55	155	36	240

The per cent actual to expected ratio shows clearly the dichotomy of underweight-low pressures and overweight-high pressures (Table III).† There is almost a 2.5 to 1 chance of the hypertensive person being overweight instead

*In the male group the lightweights had a mean blood pressure of approximately 117 mm. as compared with 121 mm. in the mediumweights. Among the heavyweights the mean systolic pressure was 124 mm. in the least heavy group, rising to over 150 mm. in the very obese. The standard errors of these means were small, thus indicating a significant difference between the blood pressures of the lightweights, mediumweights, and heavyweights. For example, using the formula $3\sqrt{(\sigma x_1)^2 + (\sigma x_2)^2}$, the difference between the lightweight pressure (116.6 ± 0.44) and the mediumweight pressure (120.9 ± 0.23) would need be only 1.5 mm. to be significant; the actual difference is 4.3 mm.

†The per cent actual to expected ratio is very simply calculated and is useful in picturing the relative effect of the extremes of one array upon the dispersion of another correlated array. We have used this technique to show the unusual influence of weight on blood pressure. For example, if, as in any random group, we find 22 blood pressures under 110 mm. to every 11 blood pressures over 140 mm., and if there were no correlation of weight to blood pressure, we should find the same ratio, 22 to 11, in any group of overweights. This is not the case; there are actually only 15 instead of 22 overweights with systolic pressures under 110 mm., and 17 instead of 11 overweights with pressures over 140 mm. This gives a per cent actual over expected incidence of 15/22, or only 68 per cent, of expected low pressures, as compared with 17/11, or 155 per cent, of expected high pressures among our heavyweight group. Simply stated, the expectancy of hypertension among heavyweights is almost 2.5 to 1.

of underweight, and the underweight person has the same chance of having a low pressure. The per cent actual to expected ratio shows the female high pressures to be even more strikingly associated with obesity than is the case with the males. Thus we find a 6 to 1 chance of the hypertensive female being overweight, while the lightweight female has almost a 4 to 1 chance of having a low pressure (Fig. 3).

It might be argued by some that the factor of age has its effect on these figures because the higher pressures and the overweights are found in the older ages, while the underweights and lower pressures are found more generally in younger persons. This criticism is partially circumvented by plotting the correlation in each age group separately and constructing this multiple correlation in the form of a three-dimensional graph, as in Fig. 4. Each row

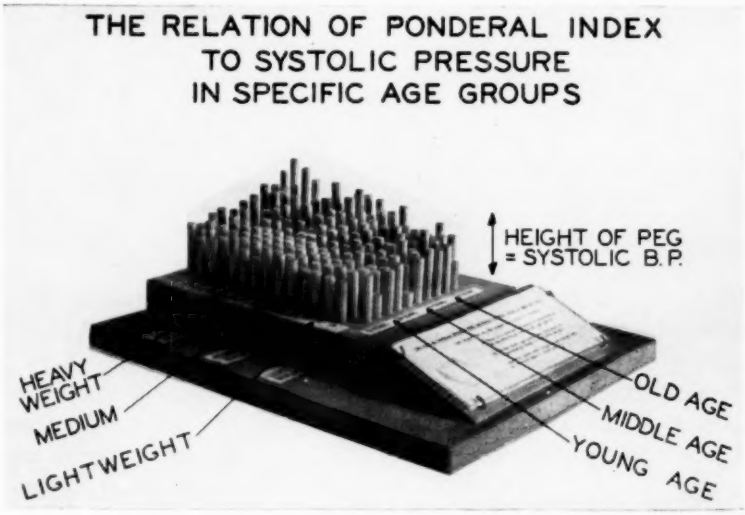

Fig. 4.—See description in text. From a model exhibited at the St. Louis Sessions of the American Medical Association, 1939.

of pegs represents an age group, from 15 to 19 years on the extreme left to 70 years and over on the extreme right. Each age group is broken down into ponderal index groups, 1.5 (the very thin persons) in the extreme front to 3.0 (the very obese) in the extreme rear. The height of the peg represents the height of the average systolic pressure for each specific age-ponderal index group.

From this multiple correlation graph we learn that in each male age group the average systolic pressure tends to rise with an increased ponderal index and that the more noticeable elevations come in the older age groups. This cannot be interpreted superficially as a pound-for-pound, millimeter-for-millimeter rise. It is cited, rather, as evidence that hypertensive persons fall most commonly in the overweight groups. The overweight groups in the older ages are more intensively affected by overweight-hypertensive males than are the young ages (Table IV). In the relatively few cases where hypertension does fall in an underweight group, it is almost always in middle-old age. In the female age groups the relationship is not as apparent. The very young and

the very old females do not follow this order. The paucity of subjects over 60 years of age lends some doubt to any interpretation of the higher pressures among the lightweight females; a few unusual subjects can easily distort the mean figure. However, an adequate explanation of this discrepancy has not yet been worked out.

TABLE IV

PONDERAL INDEX TO SYSTOLIC BLOOD PRESSURE AT VARIOUS AGES

	PONDERAL INDEX	UNDER 30 YEARS		AGES 30-39		AGES 40-49		AGES 50-59		AGES 60 AND OVER	
		M	F	M	F	M	F	M	F	M	F
Light Weight	1.7 and under	116	109	112	112	113	111	121	127	139	154
	1.8	116	110	115	111	117	116	115	118	123	152
	1.9	117	109	114	111	119	116	125	126	130	158
Medium Weight	2.0	118	112	117	112	118	119	122	120	128	149
	2.1	119	111	117	111	118	118	124	128	128	142
	2.2	120	111	116	115	120	118	123	125	134	138
	2.3	119	114	118	114	122	122	128	133	144	148
	2.4	121	115	118	118	120	124	131	133	134	143
Heavy Weight	2.5	121	113	119	115	117	126	130	132	138	128
	2.6	120	111	121	113	125	131	126	127	131	146
	2.7	121	105	124	119	125	130	133	130	138	118
	2.8	124	111	122	116	124	126	140	134	142	113
	2.9	122	–	121	123	131	131	132	144	135	138
	3.0 and over	119	110	124	122	129	129	137	138	147	149
	Total cases	1,748	635	2,387	797	1,967	563	943	232	391	84

If the incidence of high systolic pressures among the heavyweights, the lightweights, and the total male group is compared in each age group (Fig. 5), the heavyweights show an accelerating increase which tapers off at the older ages. The acceleration of incidence of high pressures in the total group lags somewhat behind the heavyweight group, but at the older ages tends to approach the heavyweight curve. While there is a rise in the incidence of high pressures among the underweights, the curve falls away from the total group curve as the age increases, and is not as apparent as in the heavyweights.

In order to visualize more clearly the effect of age, we have broken the pressures into two classifications: systolic pressures under 110 mm. and pressures over 140 mm., and each of these divisions was further broken down into two weight classes, light and heavy, for each age group. In every age group except the last the lightweight men and women show a higher proportion of low pressure than high pressure (Table V). However, as the group becomes older the ratio between low and high pressures decreases. The heavyweight men and women show a greater incidence of low pressure than high pressures up to the forty-fifth year (though much less than is found among lightweights). After age 45 this ratio is entirely reversed, there being more high pressures than low pressures among heavyweight men and women. In every age group there is a higher percentage of high pressures among the heavyweight than among lightweight men. In women this is also true except in the very young and the very old.

When the incidence of high and low blood pressures in their divided weight categories is compared on the basis of the per cent actual to expected incidence, we see very plainly the tendency toward a heavyweight-high pressure and lightweight-low pressure grouping (Table VI). Note that in each age group light-

weights show a tendency toward low pressure, and heavyweights tend to congregate in the high pressure group. Contrariwise, heavyweights show a very low ratio of low pressures and a high ratio of high pressures. Further, with an increase in age the lightweight-low pressure group shows an increased ratio. The fact that the heavyweight-high pressure ratio decreases as the group becomes older must be interpreted in the light of its relationship to the total group. While the actual incidence of high pressures increases with age among the heavyweights, the incidence of high pressures in the total group also increases, and in old age approaches the incidence among the heavyweights. Thus, the ratio

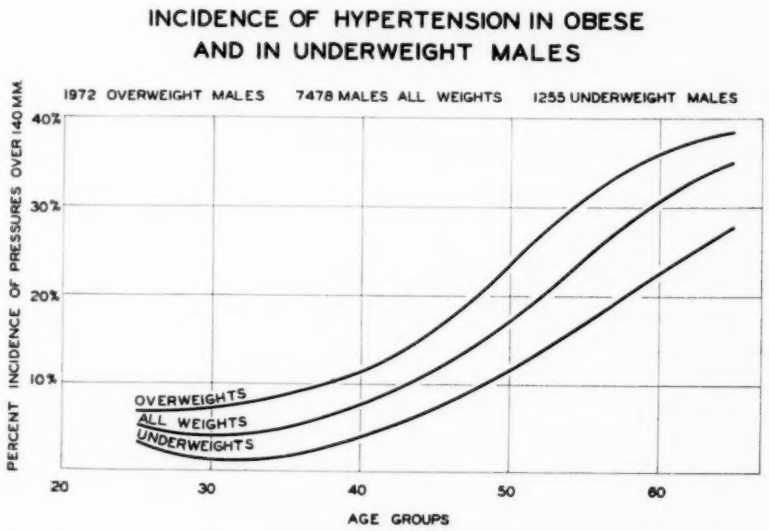

Fig. 5.—In all age groups overweight individuals show a higher incidence of hypertensive pressures.

between the two incidences will decrease. Conversely, the lightweight-high pressure incidence increment falls away from the total group curve, and the ratio between the two increases somewhat. Again, the very old female group does not conform to the general low weight-low pressure tendency—an unexplained discrepancy. In the other age groups hypertension falls more predominantly among the overweights. But it is important to call attention to the decreased correlation between obesity and hypertension as age progresses, a finding contrary to what one would expect.

THE RELATION OF PONDERAL INDEX TO DIASTOLIC PRESSURE

The male diastolic pressure, as did the systolic pressure, showed a definite increase in vascular tension with an increase in relative obesity (Table II). In the very lightweight males the modal diastolic pressure was around 70 mm. and rose fully 10 mm. as the relative obesity index rose to the heavyweight class. Whereas about 16 per cent of the lightweight males had pressures under 60 mm., less than 1 per cent of the heavyweight males had low pressures; and whereas only 5 per cent of the lightweight males had high pressures, fully 19 per cent of the heavyweight males had pressures over 90 mm.

TABLE V

PERCENTAGE INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURE AMONG LIGHTWEIGHT AND HEAVYWEIGHT MEN AND WOMEN

	AGE	20–24		25–29		30–34		35–39		40–44		45–49		50–54		55–59		60 AND OVER	
	WEIGHT GROUP	LIGHT	HEAVY	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Men	Under 110 mm.	23	23	28	14	37	16	37	17	33	17	33	14	35	12	36	10	28	8
	140 mm. and over	6	9	2	6	0	9	3	9	5	12	10	20	13	32	23	30	28	38
	Total number	170	44	261	175	234	249	158	342	129	356	105	342	68	197	39	141	40	112
Women	Under 110 mm.	58	50	52	40	47	35	46	38	46	22	41	17	23	18	29	9	9	13
	140 mm. and over	0	0	1	0	1	8	2	10	4	23	7	27	18	24	25	45	55	31
	Total number	222	14	309	45	270	83	174	122	96	150	58	144	39	74	24	53	11	16

TABLE VI

PER CENT ACTUAL TO EXPECTED INCIDENCE

PONDERAL INDEX TO SYSTOLIC BLOOD PRESSURE AT VARIOUS AGES

		7,478 MALES		2,325 FEMALES	
		LIGHTWEIGHT	HEAVYWEIGHT	LIGHTWEIGHT	HEAVYWEIGHT
Under 30 years	Low pressure	124	71	106	73
	High pressure	60	120	68	200
Ages 30-39	Low pressure	148	68	104	93
	High pressure	40	180	25	300
Ages 40-49	Low pressure	143	65	133	70
	High pressure	58	133	26	159
Ages 50-59	Low pressure	200	61	145	71
	High pressure	74	135	68	129
60 years and over	Low pressure	217	67	140	44
	High pressure	80	109	146	72

The female group showed a similar distribution. The lightweight females had a diastolic pressure of about 66 mm., which rose fully 10 mm. in the overweight group. While 20 per cent of the lightweight females had pressures under 60 mm., only about 8 per cent of the overweights had low pressures. Conversely, while only 2 per cent of the lightweight females had pressures over 90 mm., fully 20 per cent of the obese had high pressures.

TABLE VII

PER CENT ACTUAL TO EXPECTED RATIO

PONDERAL INDEX TO DIASTOLIC BLOOD PRESSURE

	7478 MALES		2325 FEMALES	
	LIGHTWEIGHT	HEAVYWEIGHT	LIGHTWEIGHT	HEAVYWEIGHT
Low pressure (under 70 mm.)	143	57	130	68
High pressure (90 mm. and over)	43	186	33	217

The per cent actual to expected ratio illustrates this relationship more clearly (Table VII). Of the lightweight males, 143 per cent of the expected will have a low pressure, while only 43 per cent will have a high pressure. Conversely, of the heavyweight males, 57 per cent of the expected will have a low pressure, while 186 per cent will have a high pressure. The female group is similarly inclined. The lightweight-low pressure group shows 130 per cent of the expected incidence, and the lightweight-high pressure group shows only 33 per cent of the incidence expected. Conversely, the heavyweight-low pressure group is only 68 per cent of the expected, and the heavyweight-high pressure group shows an incidence double that of expected. Thus, in both males and females a light weight usually is associated with low diastolic pressure and a heavy weight with high diastolic pressure.

TABLE VIII

PONDERAL INDEX TO DIASTOLIC BLOOD PRESSURE

	PONDERAL INDEX	UNDER 30 YEARS		AGES 30-39		AGES 40-49		AGES 50-59		AGES 60 AND OVER	
		M	F	M	F	M	F	M	F	M	F
Light Weight	1.7 and under	69	67	70	69	72	68	74	78	76	75
	1.8	70	68	70	69	73	70	70	70	71	87
	1.9	70	65	70	69	74	72	76	76	71	78
Medium Weight	2.0	71	67	71	72	73	74	74	76	73	83
	2.1	70	68	72	70	74	75	76	75	75	82
	2.2	72	67	72	72	75	74	74	73	78	78
	2.3	72	70	73	70	77	74	78	78	80	84
	2.4	75	69	74	73	76	75	78	77	77	84
Heavy Weight	2.5	74	69	76	75	77	78	80	81	81	76
	2.6	76	67	77	68	79	80	77	76	78	84
	2.7	76	65	78	73	80	77	83	76	81	73
	2.8	76	71	78	76	80	76	84	77	81	73
	2.9	76	–	79	74	83	80	81	82	71	78
	3.0 and over	76	71	80	76	82	79	81	82	86	86
	Total cases	1,748	635	2,386	797	1,967	563	943	232	391	84

As with the systolic pressures, it has been argued that the age factor has its effect on these conclusions, because with an increase in age we find an increase in both weight and pressure. To circumvent this criticism the ponderal index-pressure correlation was carried out in each age group where this tend-

ency of pressure to rise as weight increases was again noted (Table VIII). Again, in the aged females either the low frequency has distorted the sample or there is another factor operating which we have not as yet explained. Except for this irregularity, no matter what the age, the heavyweight person is more liable to a high diastolic pressure than either the mediumweight or the lightweight person. This evidence bears out our clinical experience: that hypertension is definitely a hazard among overweights and conversely, even more strikingly, overweight is a hazard of hypertension.

DISCUSSION

We have investigated the relationship between weight and blood pressure in a group of 10,883 individuals, residents of an urban region. An increase in weight was found to be concomitant with an increase in blood pressure. However, weight alone was found to be a poor indication of excessive fat storage because of the wide variations of obesity possible in identical weight groups. By taking height into consideration a more accurate index of relative obesity is obtained. Most existing studies are lacking in one or more of the essential criteria for a conclusive analysis, which, combined with a lack of standardization, has led to considerable divergence of opinion on the question of weight and blood pressure. In our sample we found ponderal index, an accurate measure of obesity, to vary directly with both systolic and diastolic pressure in such a manner that high pressure tended to congregate among the heavyweights and low pressure tended to be most usual among lightweights.

Since the incidence of obesity and hypertension increases with age in about the same degree, a study of mixed age groups does not measure their intrinsic correlation. Within specific age groups this obesity-hypertension correlation holds true excepting in the oldest age group. Since the degree of correlation increases with age, it is surprising to find that the oldest age group studied fails to follow the expected trend. We cannot give an explanation for this disturbing deviant correlation.

Because hypertension begins at an earlier age than obesity and because of the high incidence of familial hypertension, heredity is probably the first factor in the predisposition toward hypertension. Body build, reflecting the genetic tendency, predisposes the individual to different pressure levels, as preliminary studies indicate. It is essential to separate the obesity factor from the body-build factor. Current literature on hypertension and obesity has completely ignored their interrelation. The interrelation of body build, obesity, and hypertension will be the subject of a subsequent study.[37, 38]

CONCLUSIONS

1. In the analysis of 7,478 males and 3,405 females, weight alone was shown to be a poor measure of obesity. Consequently, a ponderal index—weight divided by height—was used as an index of relative overweight.

2. With an increase in age there is an increase in weight, as measured by ponderal index. After the age of 30 this increase in weight is probably a pathologic process.

3. With an increase in ponderal index toward obesity there is a steplike rise in both mean systolic and diastolic blood pressures in the male and female.

4. The modal systolic and diastolic pressures increased with an increase in ponderal index toward heavier weight though not as markedly as did the mean.

5. The per cent actual to expected ratio shows that the obese males have almost three times more systolic hypertension and almost four and one-half times more diastolic hypertension than do the underweight males.

6. The obese females have six times more systolic and diastolic hypertension than do the underweight females.

7. The obese males have only one-half as many low systolic pressures and only five-eighths as many low diastolic pressures as do the underweight males.

8. The obese females have only three-eighths as many low systolic pressures and one-half as many low diastolic pressures as do underweight females.

9. In any hypertensive group overweight is proportionately more common than underweight, and, conversely, in any low pressure group underweight is more common than overweight.

10. The per cent actual to expected ratio shows that among obese males high systolic pressures are two and one-half times more common than low pressures, while high diastolic pressures are three times more common than low pressures.

11. Among overweight females there are three times more high systolic and high diastolic pressures than low systolic and low diastolic pressures.

12. Among underweight males there are two and one-half times more low systolic pressures than high systolic pressures, and three and one-half times more low diastolic pressures than high diastolic pressures.

13. Among underweight females there are almost four times more low systolic and diastolic pressures than high systolic and diastolic pressures.

14. In every age group (except females over 60 years) there is an increase in mean systolic and diastolic pressures with an increase in weight.

15. Among the overweights under the age of 45 there are more low pressures than high pressures. This ratio between low and high pressures decreases with succeeding decades so that at the age of 45 there is a complete reversal and thereafter there are more high than low pressures.

16. The per cent actual to expected ratio shows that in every age group overweight males and females more often have high pressures than low pressures.

17. In every age group underweight males and females (except females over 60 years) more often have low pressures than high pressures.

18. In every age group hypertensive males and females (except females over 60 years) more often are overweight than underweight.

19. In every age group males and females with low pressure more often are underweight than overweight.

20. In general, it may be said that obesity and hypertension are frequently associated, but judgment as to the significance of this fact must be withheld until the influence of body build on obesity has been appraised.

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145 DRUNKEN DRIVERS—A BLOOD AND URINE ALCOHOL STUDY*

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IN A previous article,[1] we mentioned the fact that about 100 drunken drivers had been observed by this laboratory for alcoholic content of their blood and urine. It is the purpose of this article to present the results of this study.

We had hoped that by this time a much larger number of cases could be reported, but unfortunately, due to noncooperation, the taking of specimens on accused drunken drivers dropped off and practically ceased as a routine procedure.

Of 145 persons, we took both blood and urine specimens on 66. This gave us a chance to observe the accused person when the call to the police station was made to obtain the blood; we were, therefore, able to make a full set of observations on each person. The method used was that of the National Safety Council's Committee on Tests for Intoxication as previously explained.[2] It also gave us an opportunity to note the degree of correlation between the concentration of alcohol in the urine of these subjects and that of their blood.

There were several other advantages, chiefly from the legal standpoint. For example, one may testify repeatedly that the results of urine alcohol tests are, as a rule, close to the results of the blood test. One may state that the results run parallel after the first one and one-half hours, with the urine slightly higher, but in a close definite ratio to the blood. All this is not as definite, nor does it have the legal weight, as does being able to testify from experience with a large number of cases where blood and urine were run simultaneously. This also makes the acceptance of the urine result alone much easier. Another legal advantage to such a procedure is that one is enabled to testify from one's own experience that there is a close relationship between the results of the analysis and the observations.

Of the 145 persons, 54 had urine tests, 25 had blood tests, and 66 had both urine and blood tests.

Fig. 1 shows the correlation between the 66 blood and urine results. The first point that strikes one's attention is the peculiar bunching of the results in a rough square bounded by 0.15 to 0.25 per cent in the blood concentrations, and by 0.2 to 0.3 per cent in the urine. This peculiarity is only apparent as it becomes noticed within a few weeks of experience in running the tests. One discovers as a consequence, if the Heise method is used, that he may discard all but one dilution of the distillate as a matter of routine, and use only the one that covers the 2 to 3 mg. range in the urine and the 1.5 to 2.5 mg. range

*From the Laboratory of the Health Department of the City of Lincoln.
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in the blood. He is enabled to do this because the majority of drunken drivers fall in this class and an unknown specimen may usually be expected to contain this amount of alcohol.

A few words of explanation will make the reason for this clear. A person with a blood alcohol below 1.2 mg. per c.c. and a urine alcohol below 1.5 mg. is, as a rule, not intoxicated enough to make the effect on his driving grossly noticeable, and as a consequence, does not usually run afoul of the law unless he has an accident. Conversely, a person with over 1.5 mg. of alcohol per c.c. in his blood and over 2.0 mg. per c.c. in his urine is, as a rule, intoxicated enough to interfere with his driving to make it noticeable and invite arrest.

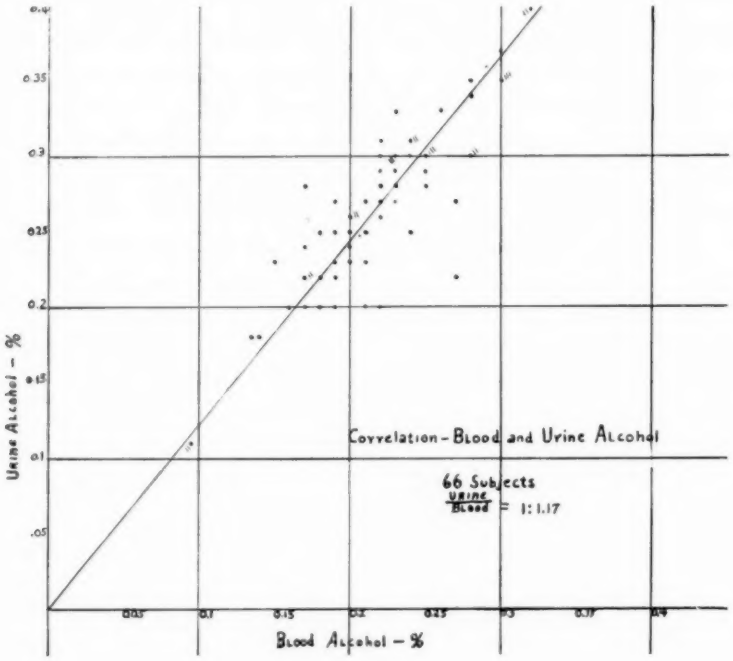

Fig. 1.

The paucity of results over 3.0 mg. per c.c. in the blood and 3.5 mg. per c.c. in the urine is likewise explainable. A person with that much alcohol in his body is nearly always too intoxicated to manipulate the controls of a car, and hence does not appear to any extent among a large group of drunken driver arrests. This is obvious when it is realized that a concentration of 3.0 mg. per c.c. in the blood of a 150 pound man is equivalent to about 12 ounces of whiskey. When one approaches one pint of whiskey, there are few, if any, individuals who will escape serious intoxication.

Table I shows the correlation of the concentration of alcohol in the urine with the degree of intoxication of 120 consecutive cases. The results, as shown by the table, agree well with those reported from other sources;[3, 4] that is, a majority of the persons fall in the 0.2 to 0.3 per cent class, and of these, 88 per cent were moderately intoxicated. By moderately, we mean the so-called

clinical stage of confusion. A representative person in this class is about 40 years old, of average weight, who has obtained his alcohol from whiskey, gin, spiked beer, or pop. He was either reported driving on the wrong side of the street, probably fast and in a manner irregular enough to draw attention; or sideswiped another car or collided with the rear end of one. Sometimes instead, he collides with the curb in rounding a corner, or runs over the curb and hits a tree, telephone, or light pole. When this same driver is on the highway, he has an accident that usually results in serious injuries or death to someone. In the city the accident is usually a minor one. The reason for the more serious results on the highway is, of course, the greater speed on the highway. He is usually arrested during the week end and usually between the hours of midnight and 4 A.M., and, as a rule, not far from a beer tavern.

TABLE I

CORRELATION OF CONCENTRATIONS OF ALCOHOL IN URINE WITH DEGREE OF INTOXICATION OF 120 SUBJECTS

URINE ALCOHOL CONCENTRATION PERCENTAGE	NO. CASES	DEGREE OF INFLUENCE BY PERCENTAGES			
		NOT INFLUENCED	SLIGHTLY INFLUENCED	MODERATELY INFLUENCED	STRONGLY INFLUENCED
0.0 – 0.10	1	1 case			
0.11 – 0.20	18	1 case	67%	27%	
0.21 – 0.30	69	1 case	11%	88%	
0.31 and over	32	1 case	0	94%	2 cases
Total	120	4 cases	16%	80%	2 cases

When brought to the station, he always insists he is not drunk and unwittingly cooperates, sometimes comically, in attempting to prove that he is not. His stock in trade alibi is that he had had two glasses of beer. When examined, his speech is usually moderately thick, his pulse usually rapid, his eyes watery and fairly often bloodshot, his pupils moderately dilated; his face is nearly always flushed and sometimes his hands and forearms as well. He may be in almost any emotional state, from morose stubborn silence to wild hilarity or tears. He walks with a decided, though slight, stagger, and if blindfolded, will always sway to some extent, usually so badly that he will lose his balance entirely. He will almost always willingly agree to the taking of a blood specimen—we have had only two who refused during the two years. His skin sensibility is noticeably deadened—venipuncture is not felt, judging from the lack of the slight flinching which a sober adult usually manifests. The result of the urine test in this stage will show about 2.3 to 2.5 mg. of alcohol per c.c., and the blood about 1.8 to 2.0 mg., corresponding in a 150-pound man to about one-half to two-thirds pint of whiskey.

All the symptoms outlined will not necessarily be present in the same person at the same time. For example, the pupils may be widely dilated, with no flush to the face, or vice versa. In other words, it is the degree and quality of those symptoms that are present that is significant, not the total number of symptoms.

The majority of specimens were run by the Heise method. The Harger titration method was used in about two-thirds of the cases to confirm the Heise result.

Our study of the 145 persons covers a period of a little over two years. During this time, constant checking of results against each other and of the methods themselves against known amounts of absolute alcohol was carried out. The two methods gave essentially the same results from the standpoint of checks against known amounts of alcohol.

The results, as shown by both tables, confirm previous results by various authors, pointing to the same conclusion; viz., that within the range of normal physiologic variations in individuals and the results of chance sampling of all classes of drunken drivers, the analysis of all body fluids can be expected to reflect about an 85 to 90 per cent true picture of the clinical effects of the amount of alcohol found.

There seems to be some evidence[5] that occasional persons may be found who do not conform to the correlation between the stages of intoxication and the concentrations of alcohol in the body fluids. The majority of this group (constituting probably 10 to 15 per cent of all people) seem to possess the ability to pull themselves together under the influence of shock of accident, arrest, excitement at the station, questioning, and examining. We have repeatedly seen this occur. We have seen a staggering drunk sober up in fifteen minutes during the course of an examination to the extent that he could walk steadily, only to fall asleep when left alone and then stagger again when aroused. There still seems to be a small percentage, probably 2 to 3 per cent, of this nonconforming group that cannot be explained on this basis. For example, an occasional person is encountered with a blood alcohol concentration of 3 mg. per c.c. or over, with little symptoms of intoxication. Jetter[5] reports one with a blood alcohol concentration of even as high as 4 mg. per c.c., who, according to him, showed little symptoms of intoxication. We saw one with 3 mg. that we were forced to describe as being practically sober.

However, we have gained an impression, which we admit seemingly has no very concrete basis, that as far as police work is concerned in drunken driving cases these persons are just as guilty of drunken driving as the person who staggers visibly. It seems somewhat significant that this kind of person seems to be subject to the same type of accidents as the more visibly affected person.

These occasional discrepancies do not invalidate the significance of the results of the tests in police work. The tests are absolute proof of the amount of unburned alcohol present in the person's body at the time the specimens were obtained, and when we prove that a person had close to a pint of whiskey or half a pint of unburned alcohol in his body at the time of arrest, we are splitting hairs when we quibble over the way he walks. He is just as much a menace as the staggering human being with the same amount in his body. The ability a person has to walk has little connection with his ability to drive a car, and when any person has between 2 and 3 mg. of unburned alcohol coursing through his brain, he is utterly incapable of controlling a car in a safe and sane manner for, in the last analysis, it is the state of a person's brain that determines his fitness to drive a car.

In a future paper we shall present the results of some animal experimentation, together with some experimentation on human beings, and a discussion of the problem of occasional puzzling results in more detail. At present we wish to emphasize that persons who apparently do not conform seem to be rare and little or no concern need be given them.

The law is slow to recognize any new scientific procedure. The states of Maine, Indiana, and Illinois have recently rewritten their laws pertaining to drunken drivers, incorporating the results of blood and urine tests as legal evidence. To the arguments of those conscientious objectors who make use of the hackneyed "constitutional rights of the individual against self-incrimination" to triumphantly prove that the use of the tests forces a person to incriminate himself, we simply refer them to the excellent discussion of the legal angles of this question by Professor Ladd.[6] The ridiculousness of this assertion is apparent when one realizes that the original and true meaning of this constitutional provision was to protect the individual from being forcibly made to confess something of which he may not be guilty. Obviously, threats, blows, arguments, or physical pain cannot change the concentration of alcohol in a person's blood. The use of this argument of constitutional right then becomes nothing but a means by which a guilty person may escape punishment and an innocent person sometimes falsely found guilty.

We can say in absolute honesty that never, in the course of our experience with these tests, has a person been falsely incriminated from the results of a test. Unfortunately, we have seen entirely too many found not guilty in spite of conclusive proof of guilt.

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THE RELATION OF LYMPHOCYTES TO THE ACTIVITY OF MYCOBACTERIUM TUBERCULOSIS*

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THE role of phagocytes in the battle of the body against disease has been a live question in the world of medical sciences for three quarters of a century, and any fact that can be brought to light on this question is of practical as well as scientific interest. Since the lymphocyte is present in all tubercular lesions, and so greatly preponderates or is the only phagocyte present in established tubercular lesions, our attention is necessarily directed toward it. The question as to the relation and mechanism of the relation of the lymphocyte to tubercular infection is a timely and poignant one.

Rather extensive literature exists on the supposed relationship of the lymphocyte to tubercular infection, but it consists largely of clinical observations and observations in connection with clinical procedures, which, while of great value in establishing the existence of a relationship, do not tell us anything of the nature or mechanism of the relationship. What experimental work has been done has in the main had as its object the test of the truth of clinical observation. Some of the experimental work has led its authors to theoretical conclusions at variance with known scientific facts and is, therefore, subject to question. No experimental work, so far as we are able to find, has shed any light on the character of the lymphocyte-tubercular relationship.

Ullom and Craig[1] first reported the fact that in acute cases of tuberculosis a relative excess of neutrophiles is found. This observation is supported by the work of Morris and Tan,[2] who found an increase in polymorphonuclear cells and monocytes in active tuberculosis. Ullom and Craig, however, point out: "The actual increase in lymphocytes seems to correspond to the increase of resistance on the part of the organism to the tuberculous infection; further study is required to confirm this deduction." Wright and King,[3] Watkins,[4] and Solis-Cohen and Strickler[5] have studied the relation of lymphocyte counts to the clinical course of tuberculosis and have concluded that when the lymphocyte count is high, the prognosis is good, and that a decreasing number of lymphocytes indicates a decreasing resistance to the infection. Webb and Williams,[6] and Webb, Newman, and Gilbert[7] are in agreement with the above conclusions. Murphy and Ellis,[8] on the basis of the results of experiments in which the lymphopoietic tissue was decreased by x-ray, believe "the lymphocyte plays an important role in the animal's resistance to tuberculosis." Hussey[9] also believes that the lymphocyte plays a part in the resistance to tuberculosis.

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Quite extensive experiments have been carried on in an attempt to show that in tuberculosis there is an inverse relationship between the monocyte and the lymphocyte; i.e., the lymphocyte is an indication of resistance to the course of the disease, whereas an increased number of monocytes indicates a loss of this resistance. The literature in this regard is extensive. Most of this work has been done by Sabin and her co-workers, Sabin,[10] and Sabin and Doan.[11] Medlar,[12] however, feels, "The factor of greatest significance in the leucocytic picture of tuberculous cases is the ratio between the polymorphonuclears and the lymphocytes. The reason for this ratio being of such great importance is that in the tuberculous lesions the polymorphonuclear represent one type (unfavorable) of pathological activity and the lymphocytes another (favorable)." Further, "It is the opinion of the author that the main function of leucocytes is to dispose of products harmful to the normal functioning of living tissue."

The mechanism by which the lymphocyte produces a resistance to tubercle bacteria has been investigated to some extent. Watkins[4] believes that the effectiveness of the lymphocyte in tuberculosis is explained by its elaboration of a lipolytic ferment which dissolves the waxy sheath enclosing the tubercle bacilli. If this is true, it is difficult to see how it could operate in a case of tuberculosis because the tubercle is a nonvascular lesion. Therefore, the bacilli would not be reached by any antibody, and, since few, if any, phagocytes other than lymphocytes are found in tubercles, there would be none to dispose of the tubercle bacilli even if they were stripped of their armor. It could hardly interfere with the growth of the bacilli because of the readiness with which tubercle bacilli grow in lymphatic tissue. Pawlow[13] has reported that lymphocytes will cause a disintegration and clumping of mycobacterium tuberculosis within two and one-half hours at 58° C. If this is the case, it could not be the important factor in lymphatic resistance because such temperatures do not occur in tubercular infections.

The question has been raised as to whether the lymphocytosis in tuberculosis is the manifestation of direct activity on the part of the lymphocyte or whether it is merely correlated with a greater resistance on the part of the individual. Smithburn[14] encouraged a lymphocytosis by injections of embryonic chick extract. He then inoculated rabbits with a virulent strain of tubercle bacillus. It was found that an inverse correlation existed; i.e., animals possessing a high lymphocyte count died sooner than the controls. Commenting on Murphy's work, Smithburn feels that the lymphatic response as indicated (by Murphy) as the cause is in reality the effect of resistance to tuberculosis. Corper[15] also has come to the conclusion that there does not seem to be a relation between the lymphocyte and the course of tuberculosis. The variance of Smithburn's results with almost universal agreement to the contrary might be explained on the basis of the intoxication produced by the chick extract.

SUMMARY OF LITERATURE

The observations so far made seem to justify the conclusions: (1) that lymphocytes play an important role in the resistance of the body to the activity of tubercle bacilli; (2) that the magnitude of the lymphocyte count is an index

to the degree of resistance of the body to the activity of tubercle bacilli, and is, therefore, of prognostical value; (3) that the polymonocyte : lymphocyte index varies with the general destructive action of the tubercle bacilli and is, therefore, high in the early acute and late stages of the disease. We have no indication as to the nature of the resistance factor of the lymphocytes, whether this factor is operative on the bacilli or whether the effect is produced in some other way, and if so in what way. The answer to this latter question must solve the paradox that while lymphocytes provide a factor in the resistance against tuberculosis, lymphatic tissue seems the most defenseless tissue in the body to the invasion and activity of tubercle bacilli.

These studies were undertaken to ascertain, if possible, (1) whether a lymphocytosis prior to infection by tubercle bacilli had any effect on the course and duration of the subsequent disease; (2) whether or not lymphocytes had any direct effect on tubercle bacilli as to virulence or in any other way; (3) to check the polymorphonuclear-lymphocyte index of Medlar; (4) granted lymphocytes play a part in the resistance to the action of tubercle bacilli, could any intimation be discovered as to the nature of the resistance factor.

MATERIALS AND METHODS

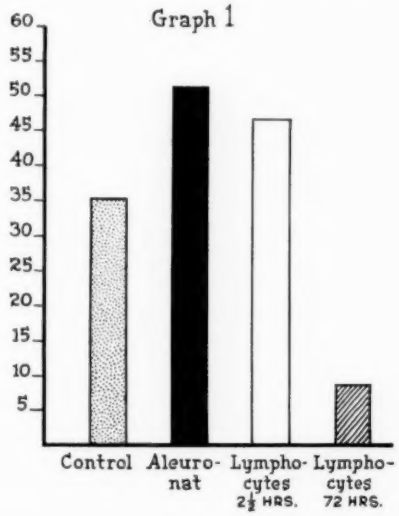
Three-hundred gram guinea pigs were the experimental animals used. They were kept in cages in a well-ventilated room of constant temperature and fed the same food; every precaution was taken to prevent intercurrent infection. The animals were divided into three groups: the first group was used as controls; the second group was untreated; the third group was injected intra-abdominally on three successive days with 5 c.c. of a sterile suspension of aleuronat to produce a lymphocytosis prior to the injection of the bacteria.

Cultures of Jamaica 22 strain of tubercle bacteria were used as the test organism because it is a strain of known high virulence. Two sets of these organisms were prepared. One set was untreated and used for inoculation into the control animals, and those in which a lymphocytosis had been produced. The other set was mixed with suspensions of lymphocytes and incubated for periods varying from two and one-half to seventy-two hours at 37.5° C. The lymphocytes for this purpose were secured by injecting massive doses (15 to 20 c.c. of aleuronat) intra-abdominally into rabbits. A week later the rabbits were injected intra-abdominally with sterile salt solution and killed immediately. The peritoneal fluid was then aspirated by means of a sterile syringe and placed in homeopathic vials. It was found that a high concentration of cells had been obtained, most of which were of the lymphocyte type. Bacteria were added to the lymphocyte suspension and incubated for varying periods of time as indicated.

Suspensions of untreated bacteria in sterile saline were made so that a 3 c.c. dose contained from 14,000 to 20,000 organisms. Three cubic centimeters of this suspension were injected intra-abdominally into each of the control animals, and each of the treated animals five days after the last injection of aleuronat. After incubation as indicated, 3 c.c. of the lymphocyte-bacteria suspension, containing approximately the same number of bacteria per dose as in the doses of untreated bacteria, were injected into animals in the third group.

The survival time of each animal was taken as the criterion of the resistance of the animal to the tubercle bacilli. Autopsies were performed on each animal at death, and the presence of tubercular lesions verified by microscopic study of the organs.

Total and differential counts of 300 cells in each case were made on one animal in the control and aleuronat groups every five days, seven animals were studied almost daily. In addition morphologic and growth studies were made of the bacteria which had been subjected to the lymphocytes in suspension.



DISCUSSION

The bacteria which had been placed in the suspension of leucocytes showed no obvious clumping or splitting-up of the bacilli. Dry preparations were made and stained by the Ziehl-Neelsen technique. No obvious changes in morphology were noted. Treated bacteria were seeded on egg-agar slants and resumed their previous growth. No evidence was seen that the lymphocytes had any effect on the morphology or viability of the tubercle bacteria, and no observations were made which would tend to uphold the findings of Pawlow.

The average survival time of the animals in each group is shown in Graph 1. The average survival time of the six control animals was 35.6 days. The longest survival time was 41 days and the shortest was 27 days. The average survival time of the 6 pigs given aleuronat previous to infection was 51.6 days, the range being between 54 and 49 days. The average survival time of 12 pigs given the bacteria-lymphocyte suspension which had been incubated two and one-half hours, was 46.8 days. The longest survival time in the group was 65 days and the shortest was 27 days. Two animals given the bacteria-lymphocyte suspension which had been incubated for seventy-two hours, died in the remarkably short time of nine days. Both were found to have died of tuberculosis infection. The viscera were almost completely covered with tubercles.

It is to be noted that while all of the animals died within the average range of longevity of guinea pigs injected with virulent tubercle bacilli, there was a

relatively longer survival time in the groups in which the tubercle bacilli had been exposed to living lymphocytes. The remarkably short survival time of the animals injected with the bacteria-lymphocyte suspension incubated for seventy-two hours was probably due to the effect of the toxins of the dead lymphocytes in addition to those of the bacteria.

The study of the blood pictures in animals in these experiments is interesting. The total white blood cell counts in the control animals showed an immediate rise which reached its peak on the fifth day and then began to drop steadily until the twenty-fifth day, beyond which no counts were made. The total count at the outset of the experiment was highest in the aleuronat animals, being 16,050, and the actual number of lymphocytes was 9,450. It is seen, however, that an immediate fall took place until the twentieth day, when a slight increase was again noted. A short rise was noted in the lymphocyte-bacteria suspension group at the end of the fifth day, with an immediate drop and then an increase in total number.

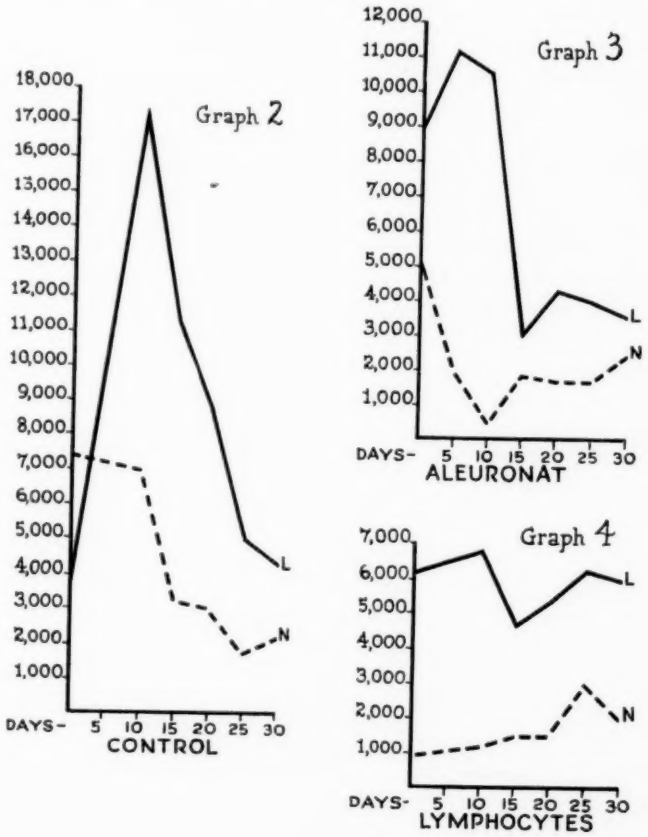
The lymphocyte count and the neutrophile count were calculated in absolute number and plotted to show the relationship of the actual number of lymphocytes to the actual number of neutrophiles. It may be seen that throughout the period of twenty-five days the lymphocytes in all cases remain higher in number than the neutrophiles. The highest number of lymphocytes in all three sets of animals was found on the tenth day, the highest actual number being found in the control animals, but with a more immediate and extensive drop in the number of cells. It is also observed that the polymorphonuclear cells tend to decrease in number during the lymphocyte rise and then begin to maintain a more or less constant level with a slight rise in number. There seems to be an almost reciprocal relationship between the lymphocytes and neutrophiles. Graphs 2 and 3 show the relationship between the actual number of lymphocytes and neutrophiles in one of the control pigs and one of the aleuronat pigs. Graph 4 shows this relationship in one of the lymphocyte pigs. These are the only two studies made on the control and aleuronat sets, whereas seven studies were made in the lymphocyte group; all tend to show the same general relations as in Graph 2. The fundamental nature of the reciprocal relationship between myeloid and lymphoid tissues has been discussed by Wiseman.

A mathematical expression of this relationship may be obtained by dividing the actual number of neutrophiles by the actual number of lymphocytes. It is found that the index for the normal pig is 0.83 (based on values given by Kleinberger and Carl in Kolmer, Boerner, and Garber's *Approved Laboratory Technic*[16]). Thus it may be seen that as the number of neutrophiles increases, the index tends upward; when the tendency is downward, it is due to increased number of lymphocytes, and the greater the resistance of the animal would be expected to be.

The control animal (Graph 2) had a rather high number of neutrophiles at the outset. The index in this case immediately fell to 0.41, and on the fifteenth day fell to 0.29, after which it began to rise and on the twenty-fifth day reached 0.60. The animal died on the thirty-seventh day of infection.

The lymphocyte count increased in the aleuronat animal to such an extent that the index at the outset was 0.62, falling to 0.05 on the fifteenth day and then rising to 0.67 on the twenty-fifth day. This animal lived forty-nine days.

The lymphocyte suspension animals were studied in more detail. In each of the seven the index was quite low, ranging between 0.015 and 0.70 at the outset. It may be further seen that there was less fluctuation in the number of cells at any particular time until shortly before the time of death. These animals, with the exception of the one shown in Graph 4, were studied until the time of death. In each case there was a gradual rise in the number of neutrophiles to the time of death, the lowest index being 0.62 and the highest being 2.



It may be further observed that the period of the second week would seem to be, on the basis of these studies, the point of greatest resistance. It is observed that following an initial rise there is a direct downward trend on the part of the lymphocytes, with an upward trend on the part of the polymorphonuclear cells.

Although an increase in monocytes is observed, it does not seem to be as constant as the neutrophilic relationship. There is further difficulty in the identification of monocytes since a "monocytoid" form of lymphocyte appears to be present in the blood of the guinea pig during tuberculous infection.

It is to be further noted that, although Spink[17] finds Kurloff Korper in monocytes during tuberculous infection of the guinea pig, we did not. Most of

the Kurloff Korper (if not all) in this study were found to be in the lymphocytes. A direct relationship between eosinophiles and Kurloff Korper was not noted.

CONCLUSIONS

A careful study of the results of these studies seems to warrant the following conclusions:

1. Lymphocytes play an important part in the resistance of the body to the action of tubercle bacilli.

2. This role is due to the activity of the active living lymphocyte. It is not due to any extracellular agglutinin, enzyme, or lysin which acts on the bacilli, but must be due to the lymphocytes disposing of the bacterial toxins, thus robbing the bacteria of their initial assault ammunition. This conclusion is further strengthened by the observation of all pathologists that tubercle bacilli seem to grow more readily in lymphatic rather than any other tissue. In this case the lymphocytes, not attacking the bacteria but disposing of their metabolic products inimical to themselves, allow a more profuse growth.

3. There is a definite lymphocytosis, the magnitude of which varies with the progress of the tubercular disease.

4. The neutrophile-lymphocyte index is highest at the onset of the disease and again at the approaching death of the animal.

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THE WELCH-LIKE BACILLUS IN HUMAN LIVER*

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IF A SMALL piece of liver is sectioned aseptically and dropped into the abdominal cavity of a dog or rabbit, death occurs in fifteen to eighteen hours.[1-7, 10] The first recorded observations on this subject were made by Mason and Davidson[1] in 1925, and their autopsy records at that time include the following: "Probably the most marked changes occur in the free liver tissue. It appears to have undergone a complete transformation, there being present no gross characteristics of liver tissue. The color is that of light chocolate while the feel is that of lung tissue, being spongy and gas containing."

It will be observed that they recorded the presence of gas in the autolyzed liver but assumed that such gas was due to the presence of the colon bacillus. However, the gas in the sectioned liver was later shown to be due to the action of Welch-like bacilli.[3, 4, 8-10] Mason and Nau[10] found that the Welch-like organism was present in the central portion of the rabbit's liver and showed that the peripheral portion of the rabbit's liver could be cultured without the presence of the organism. Emulsions of the autolyzing liver containing the organism were injected intravenously into rabbits without any ill effects. If the rabbit was killed a few minutes after the injection and subsequently placed in an incubator, the organism destroyed all soft tissue.

EXPERIMENTAL

In our present study we have cultured tissue taken from five human livers and in all we have found a Welch-like bacillus. Anaerobic cultures have been made, using pyrogallic acid with 5 per cent sodium hydroxide, but the abundance of gas generated invariably blew out the stoppers; hence we have resorted to covering the tissue with at least 1 inch of paraffin oil instead of using pyrogallic acid. We have collected and measured the gas generated and found that within twenty-four hours it is approximately seven to ten times the volume of liver cultured. Tissue taken from three different livers gave the following:

LIVER Gm.	GAS c.c.	RATIO
6.25	52	8.32
4.6	33	7.2
5.8	61	10.5

Fig. 1 is a photomicrograph of an impression smear of human liver which had been incubated anaerobically for only six hours. It will be observed that red blood cells are present in the smear, and it will be noted that the organisms growing in the human liver show an almost pure culture of the organism. There is considerable variation in the size of the organism, and such variations are com-

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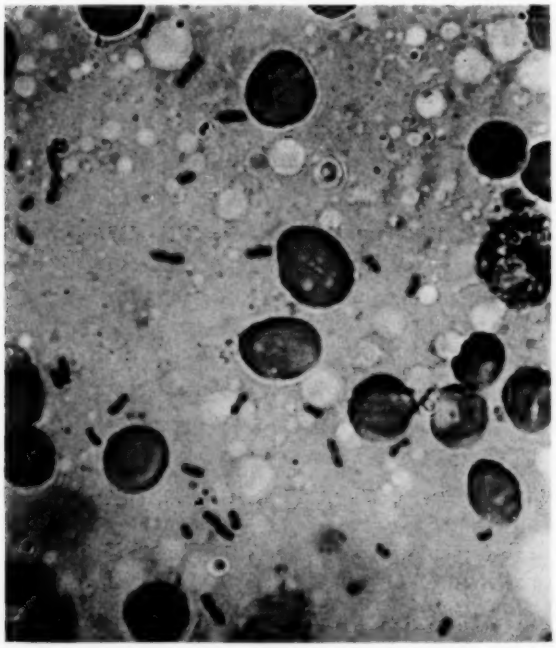

Fig. 1.—Impression smear of human liver incubated anaerobically for six hours.

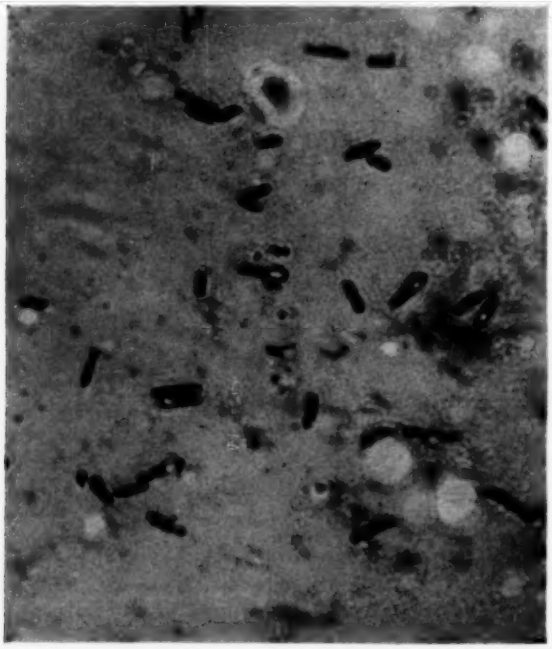

Fig. 2.—Impression smear of human liver incubated anaerobically for twenty-two hours.

monly seen in *B. welchii*. Normally *B. welchii* vary in size from 0.8 to 1.2 micron in diameter, and from 2 to 6 microns in length. It normally occurs singly or in pairs both in culture and in the tissues. Short and nearly coccoid elements are not infrequently seen. The organism shown in Fig. 1 proved to be gram-positive as is also the Welch bacillus.

Fig. 2 is a photomicrograph of an impression smear of human liver which had been incubated anaerobically for twenty-two hours. Again it will be observed that the culture is practically free of all other organisms. The average size of the organism is larger than that shown in the six-hour culture and the spore formation is quite pronounced. Apparently spore formation occurs when the carbohydrate becomes depleted. The maximum activity, measured in terms of gas formation, occurs in approximately fifteen hours and is practically completed at the end of eighteen hours. This again is limited by the amount of carbohydrate present.

We have grown the organism in milk cultures prepared by boiling the milk and cooling it rapidly to expel the oxygen. The milk is then inoculated with the organism obtained from liver culture, covered with an inch of paraffin oil, and heated at 80° C. for twenty minutes to kill all vegetative forms. The organism produces a very marked fermentation of milk.

Three cubic centimeters of the whey obtained from a milk culture were injected intravenously into a rabbit. The rabbit showed marked symptoms within thirty seconds and died within three minutes. It was then incubated for fourteen hours and had all the usual characteristics of a rabbit injected with *B. welchii*. This is known as the Welch-Nuttal rabbit test.

SUMMARY AND CONCLUSIONS

We have called this organism the Welch-like bacillus because it has the following characteristics in common with *B. welchii:* (1) It grows under strict anaerobic conditions, (2) is gas-forming, liberating seven to ten volumes of gas from liver tissue, (3) is gram-positive, (4) produces "stormy fermentation of milk," and (5) gives the Welch-Nuttal rabbit test. The bacteriologist, Kendall, considers these last two tests "presumptive tests for *B. welchii*. They are not conclusive but strongly suggestive of the presence of the organism in the material under investigation."

If the response in the human being is similar to that in other animals, it is conceivable that injuries to the human liver, which cause impairment of circulation, may afford a culture medium for Welch-like organisms and thus cause death.

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THE EFFECT OF REDUCED EVAPORATION ON THE VITAMIN CONTENT OF FRESH VEGETABLES IN REFRIGERATED STORAGE*

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THERE is ample evidence in the scientific literature that low temperatures help maintain the vitamin content of fresh vegetables, but we have found no report on the effect of reduced evaporation. In this paper evidence will be presented on the effect of storage under increased humidity and decreased air movement, both of which reduce evaporation, on the vitamin content of foods.

EQUIPMENT

Two types of domestic electric refrigerators were used in this study. Refrigerators A were of conventional construction, cooled by a coil surrounding the ice-cube compartment at the top of the cabinet. As the surface of this coil was small with respect to the cabinet area, it was necessary to maintain a larger temperature differential between the coil and the refrigerator compartment and to keep the coil sufficiently cold to get satisfactory and rapid convection cooling. This caused moisture to condense on the coil, drying the atmosphere of the cabinet, so that ordinarily the relative humidity of the compartment was below 65 per cent.

The compartment of refrigerators H was identical in size to those in refrigerators A but was divided roughly in the middle with a horizontal rubber-sealed glass shelf. The upper section was refrigerated with a coil of the same size as in refrigerators A. In the walls and floor of the lower compartment a long continuous refrigeration coil was imbedded. Because of the large surface of this coil it was necessary to maintain only a slight temperature differential between the coil and the compartment, reducing the condensation so that ordinarily the relative humidity of the lower compartment was 93 per cent or more.

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In all experiments the temperature at the center of each refrigerator at a distance of 25 cm. from the floor was held at about 41° F. This position was 15 cm. below the glass shelf in the H refrigerators and 60 cm. below the ceilings of all the refrigerators. In all experiments comparisons were made between food substances placed in the humid lower compartment of refrigerators H and in corresponding positions in refrigerators A. Temperature, location, and time were substantially the same in all instances except as noted below; essentially only humidity and convection (air movement) were different. Differences in results could, therefore, be ascribed to these two factors.

The rate of evaporation of moisture from foods stored in the A refrigerators averaged some four times that of foods stored in the H refrigerators. Much less moisture was lost by foods placed in covered metal containers on the floor of refrigerators A than when placed on open shelves, because convection was lowered and the humidity of the air in these containers rose to about 93 per cent. Foods in similar vegetable containers and in like positions in refrigerators H lost least moisture because convection was further reduced and the relative humidity was raised above 98 per cent. Evaporation from foods stored in the vegetable containers in A refrigerators was substantially two and one-half times more rapid than in containers in corresponding positions in H refrigerators. This was due in part to a difference of 8° F. in the temperature of the interior of the containers, as in refrigerators A cooling was dependent upon convection from the coils at the top of the refrigerator.

MATERIALS AND PROCEDURE

Green vegetables were selected because they are especially active biologically, are sensitive to changes in humidity and air movement, are particularly good sources of the unstable vitamins, and are commonly stored in domestic refrigerators. Chemical, photometric, and biological tests were made to determine the rate of loss of the unstable vitamins A, B_1 (thiamin), and C (ascorbic acid) in these vegetables. Because of the extreme sensitivity of ascorbic acid, and because of the convenience and reliability of the chemical titration procedure by which ascorbic acid is determined, data from measurements of this vitamin predominate. For the chemical and photometric tests, vegetables of the best available quality were secured from local retail markets. For the biological tests on vitamins A and B_1, green lettuce, and for the biological tests on vitamin C, parsley, were secured daily, freshly picked from the same greenhouse. All tests were made in the late summer and fall of 1938.

Each lot of samples was placed in a small dish in the refrigerators. The dishes were weighed to 0.01 Gm. before and after loading with samples, and again upon removal from storage. Chemical determinations of the vitamin content were then made, and the results compared with data similarly obtained on samples tested before storage. Results are expressed in relation to the original weight of the samples, which were refrigerated for three, four, five, and seven days. Vegetables stored for more than seven days in the A refrigerators were generally unpalatable.

ASCORBIC ACID, CHEMICAL ASSAY

The amount of ascorbic acid in lettuce was determined by Tillmans' procedure,[1] as modified by Mack and Tressler.[2] In testing vegetables other than lettuce, however, this procedure was departed from to the extent that the pulverized and precipitated cell fragment material was not separated from the extract before titration because it did not interfere with the determinations and it allowed a more rapid determination. Except in one instance, ten or more lots of samples were tested for each position or storage interval, and the loss in ascorbic acid was determined separately for each. From these data the mean averages and variations were calculated. The data from 537 determinations are given in Table I.

TABLE I

EFFECT OF HUMIDITY AND AIR MOVEMENT ON ASCORBIC ACID LOSS
(Average percentage losses and mean deviation based on original weight.)

POSITION		REFRIGERATORS A	REFRIGERATORS H	REFRIGERATORS H (VEG. CONTAINER)
AVERAGE HUMIDITY		65 per cent	93 per cent	98 per cent
VEGETABLE	DAYS STORED	PERCENTAGE LOSS IN ASCORBIC ACID		
Lettuce	1	26.5 ± 13.3	14.3 ± 16.6	20.4 ± 16.3
	3	55.1 ± 4.1	36.7 ± 10.2	22.4 ± 16.3
	7	48.0 ± 19.4	38.8 ± 19.4	33.7 ± 13.3
	Avg.	43.2 ± 12.5	29.9 ± 15.4	23.5 ± 15.3
Spinach	4.5	77.4 ± 6.8	67.6 ± 13.6	62.4 ± 15.0
	7	82.4 ± 2.6	58.6 ± 10.0	66.4 ± 7.1
	Avg.	79.9 ± 4.7	63.1 ± 11.8	64.4 ± 11.0
Parsley leaves	4	17.4 ± 6.2	8.0 ± 8.9	3.8 ± 10.2
	7	31.8 ± 5.0	21.0 ± 7.9	15.4 ± 7.1
	Avg.	24.6 ± 5.5	14.5 ± 8.4	9.6 ± 8.5
Snap beans	3	30.8 ± 12.8	24.4 ± 12.8	28.2 ± 12.8
	7	50.0 ± 6.4	32.1 ± 6.4	35.9 ± 12.8
	Avg.	40.0 ± 9.6	28.2 ± 9.6	32.0 ± 12.8
Shelled lima beans	4	35.0 ± 10.6	31.5 ± 9.8	19.3 ± 8.3
	7	54.8 ± 4.7	29.2 ± 7.9	28.0 ± 8.7
	7[a]	39.4 ± 5.5	10.2 ± 8.7	4.7 ± 7.1
	Avg.	43.1 ± 6.9	23.6 ± 8.6	17.3 ± 8.0
Shelled peas	5[a, b]	12.9 ± 4.8	4.5 ± 3.4	1.3 ± 7.2
	7	36.7 ± 6.9	0.2[c] ± 1.8	6.8 ± 4.9
	Avg.	31.9 ± 5.8	1.0 ± 2.2	5.4 ± 6.0
Average	4	38.1 ± 7.6	28.8 ± 9.8	22.9 ± 11.6
	7	49.3 ± 7.6	28.4 ± 9.0	29.1 ± 8.8
	Avg.	43.6 ± 7.6	28.6 ± 9.4	26.0 ± 10.2

[a]This series was run earlier in the season than other series of the same vegetables.
[b]Only three runs; average weighted accordingly.
[c]Gain.

Although there were variations in the data on the same vegetables and on different vegetables, there was a consistently greater destruction of ascorbic acid in the vegetables stored in the A refrigerators than in vegetables stored in the same position in the H refrigerators or in vegetables stored in the vegetable container on the floor of the H refrigerators. Concurrent with the marked reduction in evaporation from the vegetables stored in the vegetable container in the H refrigerators, there was a tendency for the destruction of ascorbic acid to be less rapid during the first four days and more rapid during the next three

days than when these vegetables were stored outside the container but in the same refrigerators. At the end of seven days, however, the destruction was substantially the same.

The data in Table I are presented in condensed form in Table II and Fig. 1. In Table II the data are treated by the use of index numbers to make the comparison clearer. The index numbers indicate that the destruction of ascorbic acid averaged over 64 per cent more rapid in refrigerators A than in the more humid refrigerators H. The least difference was shown by spinach, which lost ascorbic acid 27 per cent more rapidly in refrigerators A. Supplementary experiments indicated that destruction of ascorbic acid in closed vegetable containers on the floor of refrigerators A also proceeded at a rate substantially more rapid than in refrigerators H, either in the open or in similar containers.

TABLE II

SUMMARY OF AVERAGE ASCORBIC ACID LOSSES IN GREEN VEGETABLES UNDER DIFFERENT STORAGE CONDITIONS

POSITION	PERCENTAGE LOSS			INDEX NUMBERS		
	REFRIGERATORS A	REFRIGERATORS H	REFRIGERATORS H (VEG. CONTAINERS)	REFRIGERATORS A	REFRIGERATORS H	REFRIGERATORS H (VEG. CONTAINERS)
AVERAGE HUMIDITY	65%	93%	98%	65%	93%	98%
Lettuce	43.2	29.9	23.5	144	100	78.5
Spinach	79.9	63.1	64.4	127	100	108
Parsley	24.6	14.5	9.6	170	100	66
Snap beans	40.4	28.2	32.0	143	100	113.5
Shelled limas	43.1	23.6	17.3	183	100	73.5
Shelled peas	31.9	1.0	5.4	3,190	100	540
Averages	43.85	26.7	25.4			
Index No.	164.3	100.0	95.1			
Index No.	172.6	104.9	100.0			

This extensive series of determinations indicates the hitherto unemphasized importance of the effect of evaporation on the ascorbic acid content of fresh green vegetables and demonstrates the importance of maintaining such foods in an atmosphere of high humidity and low air movement, as well as at a low temperature, to preserve their ascorbic acid content.

ASCORBIC ACID, BIO-ASSAY

The more extensive destruction of ascorbic acid in green vegetables stored in refrigerators A than in refrigerators H was confirmed by bio-assay[3] on two sets of young scorbutic guinea pigs.

Each day during a period of four weeks an equivalent of 0.7 Gm. (fresh weight) of parsley, which had been stored respectively in refrigerators A and H for six days, was fed to each animal as a supplement to the scurvy-producing diet. At the end of the test period the animals fed the extensively dehydrated parsley stored in the A refrigerators had lost 1.9 per cent in weight, while those fed parsley stored in the H refrigerators had gained 4.8 per cent in weight. The experiments from which these results were derived, however, were not sufficiently extensive to be more than corroborative of the chemical findings.

VITAMIN A, PHOTOMETRIC

The amount of carotene (provitamin A) destruction was estimated, using a recording spectrophotometer[4] which plotted the reflectance curves of vegetables before and after storage in the A and H refrigerators and in the vegetable containers in the H refrigerators. These spectra were compared with those of alpha and beta carotene and oxidized carotene.

The results indicated that there was more destruction of the carotene in lettuce and carrots stored in the A refrigerators than in the more humid H refrigerators. No difference in carotene destruction was noted with respect to spinach, tomatoes, and Swiss chard.

Interpretation of results was made difficult because the spectra for the carotenes and the chlorophylls overlap in the regions of greatest absorption. An instrument which would read in the region of the ultraviolet maximum for carotene is needed.

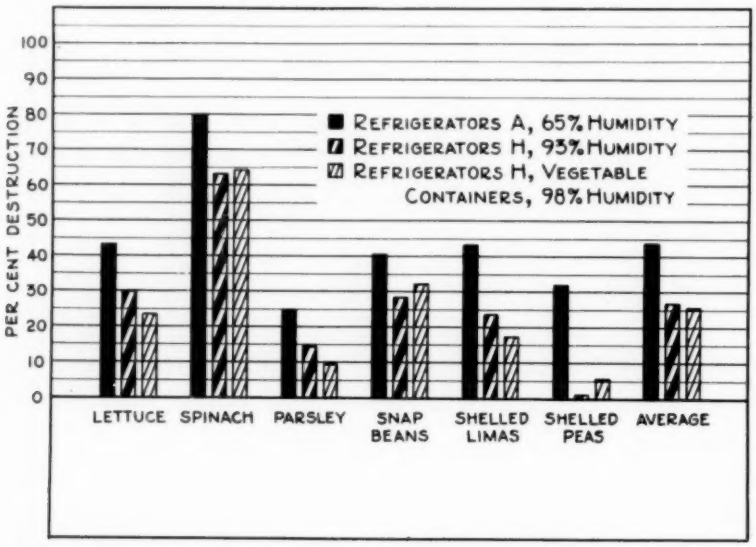

Fig. 1.—Average destruction of ascorbic acid in refrigerated green vegetables as influenced by evaporation.

VITAMIN A AND B_1 BIO-ASSAYS

Biological tests were performed to determine qualitatively whether vitamins A and B_1 like ascorbic acid show a tendency to be destroyed more rapidly at lower humidities. One group of rats made deficient in vitamin A[5] was fed a daily supplement of 60 mg. of lettuce (fresh weight) that had been stored for four days in the A refrigerators. A similar group was fed the same amount of lettuce that had been stored for four days in the H refrigerators.

One group of rats made deficient in vitamin B_1[6] was fed daily 1.00 Gm. of lettuce that had been stored for four days in the A refrigerators, while a similar second group was fed daily 1.00 Gm. of lettuce stored for the same period of time in the H refrigerators. The lettuce was comminuted and mixed into the diet.

In both instances the animals fed the lettuce stored in the H refrigerators showed better weight increases, indicating that the vitamin A and vitamin B_1 of lettuce is better preserved when vegetables are so maintained in refrigerated storage that evaporation is minimized.

SUMMARY AND DISCUSSION

An extended series of determinations indicated that the maintenance of vitamin C (ascorbic acid) in fresh green vegetables is considerably improved by storage at a high humidity and low air movement. During storage in domestic refrigerators the destruction of this vitamin proceeded at a rate averaging some 64 per cent greater for six green vegetables stored at an average humidity slightly below 65 per cent and with considerable air movement than when stored at the same average temperature (41° F.) but at an average humidity of about 93 per cent and with low air movement. Biological and photometric measurements indicated that vitamin A and vitamin B_1 (thiamin) as well as vitamin C (ascorbic acid) were better preserved when vegetables were stored in more humid atmospheres.

Supplementary studies showed that the storage of fresh vegetables at high humidity and low air movement markedly retarded wilting and consequent wilting plasmolysis. It is customary for wilting plasmolysis to accelerate the oxidation of the cell constituents and impair the natural resistance of the plant to microbial invasion. This may explain why vegetables maintained in a humid and quiet atmosphere show better maintenance of vitamins and overall freshness.

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FURTHER STUDY OF THE Vi ANTIBODY CONTENT OF THE SERA OF TYPHOID PATIENTS AND CARRIERS

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THE development of Vi antibody to a titratable degree in the sera of typhoid patients has been shown to be sporadic and unpredictable (Almon and Stovall;[1] Almon, Read, and Stovall;[2] Felix, Krikorian, and Reitler[8]). Its occurrence in the sera of chronic carriers, on the other hand, seems to be a much more common occurrence. Felix[6] reports that the proportion of carriers in whose sera the antibody may be demonstrated is high enough to make it feasible to use this characteristic in the location of carrier suspects. Bhatnagar, Speechly, and Singh,[3] and Pijper and Crocker[9] report that all carriers who are shedding organisms may be shown to have the Vi antibody. Although the results reported in this paper do not differ materially from those of other workers, the additional number of sera studied may add to the statistical value of the figures.

We have tried to choose a method both sensitive and accurate. The earlier work on the titration of the Vi antibody was done by absorbing the sera of their O and H antibody content and testing for the remaining Vi with a culture known to be highly agglutinable by this antibody. More recently, three direct methods, somewhat similar in principle, have been proposed for demonstrating this antibody. One of them, that of Detre,[5] consists essentially of the selection, by alternating agglutination and plating procedures, of a culture of *Eberthella typhosa* completely inagglutinable in typhoid O antiserum; and of the removal of the flagella from a suspension of this culture by repeated washings in saline. The resulting suspension is not agglutinated by O or H antibodies, but fully retains its sensitivity to the action of the Vi antibody. Bhatnagar, Speechly, and Singh[3] found what they described as a Vi variant, i.e., a culture which possesses no H antigen and shows little O and remains stable in this condition. They used it for the direct titration of the Vi antibody and found frequent control and rejuvenation procedures unnecessary. Their method, therefore, is like that of Detre except that their culture is stable in the Vi condition. The third method, described in detail by Felix,[7] makes use of carefully selected strains of organisms, but dispenses with removing the flagella, and standardizes their Vi agglutinability with a "provisional standard serum." It is this method which has been used by Felix for the titration of carriers' sera.

Since the work reported in this paper was begun before the details of Felix's method had been published and before the existence of Bhatnagar's culture had been announced, the method of Detre,[5] slightly modified, was the one employed. The cultures to be used were selected, as in the original method, by plating from the supernatant liquid of an agglutination tube in which those organisms

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TABLE I

A COMPARISON OF THE RESULTS OF TITRATING SERA FOR VI ANTIBODY FOLLOWING ABSORPTION AND BY THE METHOD OF DETRE

CASE NO.	TIME SINCE ONSET OF ILLNESS	TITER BY DETRE METHOD	TITER FOLLOWING ABSORPTION
Severe cases			
9	44 days	0	0
11	18 days	0	0
20	49 days	1:40	0
	95 days	0	0
28	6 months	0	0
55	8 months	0	0
Mild to moderate cases			
1	22 days	0	0
	37 days	0	0
	59 days	0	0
2	4 days	0	0
	41 days	0	0
3	21 days	0	0
5	45 days	0	0
6	8 days	0	0
	17 days	0	0
18	28 days	0	0
	42 days	0	0
	50 days	0	0
19	68 days	1:40	1:40
30	43 days	0	0
40	15 days	0	0
	21 days	0	0
	27 days	0	0
43	27 days	1:80	0
47	6 days	0	0
Carriers			
17	Not known	1:160	0
	Not known	1:160	0
22	28 years	1:40	0
23	35 years	1:40	0
31	No history of typhoid	0	0
37	4 years	1:40	0
38	20 years	1:40	0
39	No history of typhoid	0	0
42	17 years	1:80	1:80

sensitive to the O antibody had agglutinated and settled out. The organisms remaining in the supernatant liquid were, therefore, chiefly of the O resistant type. From the plates a number of colonies were transferred to slants and tested for their O agglutinability. These procedures were repeated until several strains completely inagglutinable in potent O antiserum were found. The cultures thus selected were seeded on plain agar plates, and after twenty-four hours the resulting growth was washed off with saline. The suspensions thus obtained were centrifuged and the sediment was resuspended in fresh saline, this being done six times. The final suspensions were tested for their O, H, and Vi agglutinability with highly potent antisera maintained for this purpose. Those suspensions which were totally inagglutinable in 1:40 and 1:80 dilutions of the O and H sera and were strongly agglutinated by the Vi serum were saved for subsequent tests. (This method of testing for the complete removal of the H antigen has been entirely satisfactory, and seems simpler than the India ink method included in Detre's original description.) Such suspensions, if not too highly diluted, maintained their Vi character for periods of at least two weeks in the refrigerator.

TABLE II

VI, O, AND H ANTIBODIES IN PATIENTS' AND CARRIERS' SERA

	CASE NO.	TIME SINCE ONSET OF ILLNESS	VI TITER	O TITER	H TITER
Severe cases					
	59	28 days	1:40	1:5,120	1:5,120
		38 days	0	1:2,560	1:160
	61	9 days	0	1:320	0
		21 days	1:80	1:80	0
(Fatal)	65	22 days	1:40	1:1,280	1:160
(Fatal)	66	16 days	0	1:640	1:2,560
Mild to moderate cases					
	35	24 days	0	1:640	1:640
		28 days	0	1:640	1:640
	53	?	0	1:160	1:80
	54	?	1:160	1:5,120	1:2,560
	56	7 days	0	1:160	0
		28 days	0	1:160	1:160
	57	10 days	0	1:1,280	1:160
		28 days	0	1:640	1:160
	58	20 days	0	1:320	1:320
		35 days	0	1:320	1:160
	60	10 days	1:40	1:1,280	1:1,280
		18 days	1:40	1:640	1:640
	62	15 days	0	1:40	0
		25 days	0	1:160	1:40
	63	28 days	0	1:640	0
		31 days	0	1:640	0
	64	14 days	0	1:160	0
Carriers					
	44	10 years	0	1:160	1:80
	67	6 years	1:40	1:1,280	1:40
	68	29 years	1:80	1:5,120	1:160
	69	No history of typhoid	1:80	1:2,560	1:1,280
	70	4 years	1:80	1:160	1:640
	71	No history of typhoid	1:40	1:640	1:160
	76	No history of typhoid	1:80	1:640	1:160
	77	3 years	1:80	1:1,280	1:160
	78	28 years	1:320	1:640	1:5,120
	79	8 years	1:80	1:320	1:640
	80	2 years	1:80	1:640	1:160
	81	32 years	1:80	1:640	1:640
	82	40 years	0	1:80	1:640
	83	37 years	1:160	1:80	1:160
	26	2 years	1:160	1:80	1:320

The authors are indebted to Dr. E. S. Sanderson, of the University of Georgia, for collecting and sending the sera and histories of 11 cases reported in this table, and to Dr. Forsbeck, of the Michigan Department of Health, for similarly furnishing us with 13 sera from carriers.

The experiments here reported deal with (a) the retitration by the Detre method of what remained of the sera reported upon in a previous paper (Almon, Read, and Stovall[2]); (b) the titration of sera from 14 patients more recently made available; (c) the titration of sera from 15 carriers also just recently made available.

A comparison of the titers of the Vi antibody, as determined by the absorption method and by the method of Detre, is presented in Table I. The inagglutinability of the Vi suspension in O and H antisera was controlled in every series of agglutination tests by setting it up in 1:40 and 1:80 dilutions of rabbit antisera having high O and H titers. The figures show that the presence of Vi antibody, as demonstrated by the classical means, was confirmed in each case by the method of Detre. This was true also in some tests on rabbit antisera, not included in the table. Not only was the presence of the

Vi antibody confirmed by the new method in those sera which had been shown by the old method to contain it, but 6 human sera and 5 of a total of 31 rabbit sera tested, which had given negative results by the absorption method, were shown by the more direct method to contain the antibody, thus giving evidence for the greater sensitivity of the direct method. In the light of these results the newer method was the only one employed in the subsequent work.

The results obtained by the titration of the sera recently acquired from cases and carriers are given in Table II. In order to make this report parallel with previous ones, the titers for O and H antibodies are also included.

From Tables I and II in this paper and from Table II in the paper published previously (Almon, Read, and Stovall[2]) the essential findings concerning the Vi antibody content of human sera are brought together in summary form in Table III. These data substantiate our previous conclusion that the appearance of the Vi antibody is not demonstrably correlated with severity of symptoms of typhoid infection nor with any particular stage of active infection. The correlation with the existence of the carrier state seems to be more definite. It is possible that even a greater percentage of positive results among carriers' sera might have been obtained if all of these originally studied had been available for retitration by the Detre method. In the original study only two of eleven carriers were shown to have the antibody in their blood, but upon retitration of those samples (seven in number) which had not been used up, all but two were shown to contain the Vi antibody. Of those which were not available for restudy, one had been shown by the absorption method to contain Vi antibody. This leaves two still in question. But at best our figures for the occurrence of the antibody among carriers would be somewhat lower than those of Felix,[6] of Bhatnagar and his co-workers,[3] and of Pijper and Crocker.[9]

TABLE III

SUMMARY OF PRESENT AND PREVIOUS WORK CONCERNING THE OCCURRENCE OF THE VI ANTIBODY IN HUMAN SERA

	NO. OF PATIENTS INVESTIGATED	NO. POSITIVE FOR VI	PER CENT POSITIVE
Severe cases	14	6	43
Mild to moderate cases	26	9	34
Carriers	26	20	77

It is conceivable that with more refined methods, tissue reactivity to the Vi antigen may be detected in still more individuals who have been infected with the organisms, since nearly all of the evidence points to the occurrence of this antigen in most freshly isolated cultures from patients and carriers alike (Felix, Krikorian, and Reitler;[8] Craigie and Brandon;[4] Welch and Mickle;[10] Almon, Read, and Stovall[2]). This detection might be accomplished by the use of lower dilutions of the sera if complications due to factors other than the Vi antibody could be ruled out. Felix and his co-workers in all of their work dealing with this antibody have reported titers as low as 1:5, but we have never been able to satisfy ourselves in our own work that results with such low dilutions were reliable. However, the procedure of measuring cir-

culating antibodies is at best a crude one for the study of immunity. The fixed tissue antibodies in this disease remain entirely uninvestigated.

SUMMARY

By a direct titration method 15 of a total of 40 typhoid patients investigated and 20 out of 26 typhoid carriers were found to have demonstrable amounts of Vi agglutinin in their blood. It is concluded that the correlation between the presence of this antibody and the existence of the carrier state is significantly high, but no attempt is made at interpretation of this phenomenon.

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CACHEXIA RESPONDING TO EXTRACT OF THE ANTERIOR LOBE OF THE PITUITARY*

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SIMMONDS[1] described a cachexia due to destructive lesions of the anterior lobe of the pituitary gland. He originally described the syndrome as one of premature aging, atrophy of the genital organs with loss of sexual characteristics, and loss of hair. He believed the etiology to be that of a complete destruction of the pituitary gland by embolism. Later, however, patients were described in whom there were destructive lesions of the pituitary gland due to neoplasm or aneurysm, and in many no cause has been found.

Lisser and Escamilla[2] analyzed the symptoms of 134 clinical cases of Simmonds' disease, of which 69 were verified. The symptoms found in the verified cases reveal that females predominate over males 2 to 1. There was marked loss of weight, with an average weight of 91 pounds; in fact, weight loss is

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suggested as a cardinal symptom. Asthenia was present in 83 per cent; psychic apathy and somnolence were present in 48 per cent; anorexia in 33 per cent; and vomiting in 30 per cent. Diabetes insipidus was described in 13 per cent; sensitivity to cold was present in 41 per cent; amenorrhea in 52 per cent. Libido was lost by 58 per cent of the males. Premature aging occurred in 46 per cent and dry skin in 56 per cent. Loss of axillary and pubic hair was observed in 38 per cent. The basal metabolic rate averaged –31, and a low body temperature was found in 78 per cent.

A careful review of the literature fails to reveal case reports that can be closely correlated with the following report of a man who has been under observation since December 26, 1937, with the possible exception of a case report by Osgood.[3]

Osgood observed a female, who, although she had a voracious appetite, persistently lost weight, and became greatly emaciated. She had an achlorhydria, low basal metabolic rate, and a persistent diarrhea. She was treated for a time with antuitrin-S. Four years later she died from pulmonary tuberculosis, but at autopsy the pituitary gland was found to be normal. Osgood reports this case as an instance of questionable pituitary cachexia.

L. W., a 64-year-old white laborer, entered Cook County Hospital December 26, 1937. His immediate complaints were diarrhea, loss of weight, and edema of the feet, which had come on suddenly three weeks previously. He was irritable and after a short stay left the hospital. He was readmitted on January 27, 1938, with the same complaints. He stated an operation had been performed in 1915 for a gastric ulcer (according to his history, a part of the stomach had been removed and a new opening made). However, repeated x-rays of the stomach and direct gastroscopy gave no evidence of a gastroenterotomy. Since the time of his operation there had been no recurrence of his gastrointestinal symptoms. The edema of the feet had been noticed at various times during the past three years; in fact, during that period he had been treated at a dispensary as a chronic cardiac patient. The remainder of the history was essentially negative.

At the time of examination he was pale, emaciated, weak but not acutely ill. His temperature was 98.2° F.; pulse rate 48, respiratory rate 9, and blood pressure 126/80. He showed evidence of marked weight loss. An examination of the chest and abdomen revealed no abnormalities. Reflexes were normal. A slight edema of the ankles was present, which, however, disappeared with bed rest.

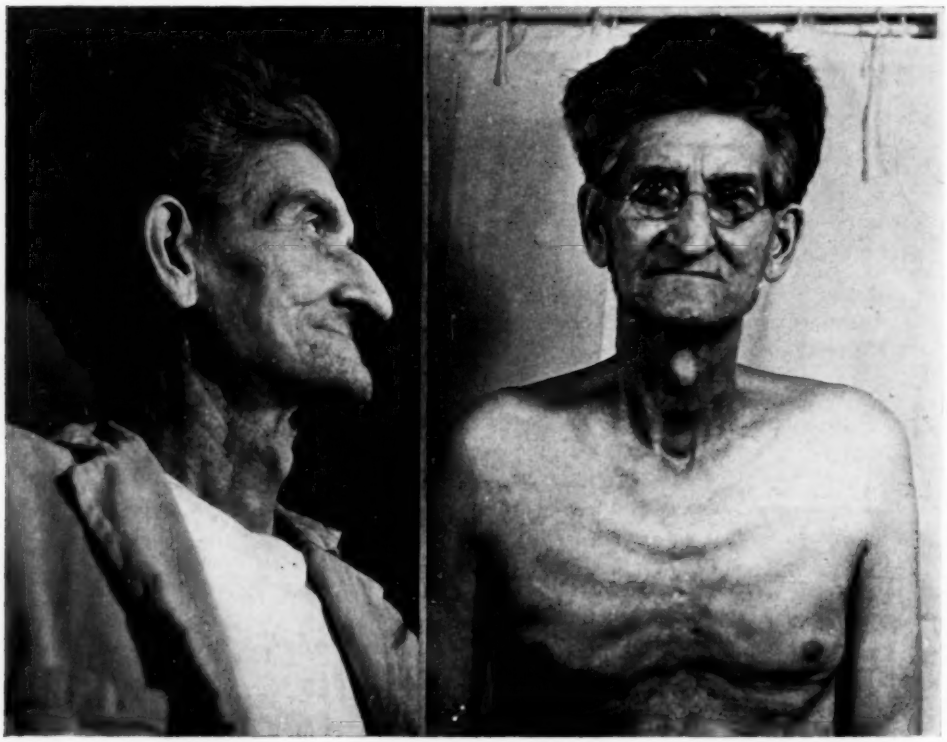
He became progressively weaker, with a persistent diarrhea of from 5 to 12 stools a day. These stools were yellow, watery, and of low specific gravity, but contained no blood. There was a complete achlorhydria after an ordinary Ewald test meal. Repeated x-rays of the gastrointestinal tract failed to reveal any abnormality. Direct gastroscopic examination by Dr. Schindler revealed a few superficial erosions upon a reddened mucosa. A proctoscopic examination showed a reddened mucosa. The basal metabolic rate was –34. Blood and spinal Wassermann tests were negative. There were no abnormal constituents in the urine. Blood chemistry showed nonprotein nitrogen 25, urea 15.41, uric acid 2.5, cholesterol 160, sugar 86, total protein 6.83, albumin 4.37, globulin 2.46, and ratio 1.77.

Shortly after entering the hospital a severe glossitis developed. Neither the glossitis nor the diarrhea was improved by the administration of nicotinic acid or intramuscular liver extract.

During his entire stay in the hospital his appetite was better than average, and, although he was given a high caloric diet, the weight loss continued. The basal metabolic rate remained low, averaging from –26 to –34. From a normal weight of 180 pounds his weight gradually decreased to 96 pounds. By this time he had become so weak that he was hardly able to lift his hands.

Various forms of therapy were tried during the period up to October, 1938. During this time he was treated with high caloric and high vitamin diets, intramuscular injections of liver extract, and, at various times, thyroid, insulin, and nicotinic acid.

During this period there had been considerable discussion as to the cause of his illness, and it was finally decided that although he did not present a typical picture of a Simmonds' disease patient, he should be treated for the disease. Accordingly, beginning October 19, 1938, he was given daily 1 c.c. of extract of the anterior lobe of the pituitary gland* intramuscularly, contained no water-soluble vitamins. By April 8, 1939, he had gained 70 pounds, his weight being 161 pounds. Shortly after beginning administration of the extract of anterior lobe of pituitary the patient began to feel better. His diarrhea decreased, and after thirty days he began to gain weight and strength. By May, 1939, he weighed 167 pounds and his basal metabolic rate was –10.



A. *B.*

Fig. 1.—*A*, December, 1938. Weight about 100 pounds. *B*, May, 1939. Weight about 160 pounds.

The pituitary extract was discontinued in May, 1939. The patient continued well until October 15, 1939. After a loss of 9 pounds and a recurrence of the diarrhea, he was given 1 c.c. of the gonadotropic hormone of the pituitary gland daily for thirty days, with prompt relief of the diarrhea and return to his previous weight level. He is now up and about the ward, although he has not fully regained his strength. Otherwise he appears to be perfectly normal.

The gonadotropic hormone was not continued because of the belief that it was impossible to procure an extract free of the other hormones.

The history of a patient seen in private practice seems to be a partial parallel of the preceding case.

*The extract of the anterior lobe of the pituitary gland, "polyanson," of Armour & Company was used throughout. The method of preparation of polyanson should destroy all water-soluble vitamins.

Mrs. T. R., was seen on August 14, 1939. She had recently moved to Evanston from Colorado. Her initial complaint was that of weakness, loss of weight, and emaciation. She stated that during the past five years she had continuous diarrhea of from three to numerous liquid stools daily. This diarrhea had been present at various times for the past fifteen years. Menses were irregular, the quantity greatly decreased and usually accompanied by pain.

An examination revealed an emaciated white woman, 44 years of age. Her facies were striking; she was wrinkled and looked many years older than the age given. She had the peculiar wizened expression seen in the hypopituitary dwarf and a slight glossitis. Her weight was 92 pounds.

Examination of her blood showed hemoglobin 14.5, red blood cells 4,800,000, white blood cells 6,050, total nonprotein nitrogen 29.5, glucose 91, cholesterol 213, and chlorides 485. Basal metabolic rate was –7. Gastric analysis revealed free hydrochloric acid 6; total acidity 22. X-ray of the gastrointestinal tract was entirely negative.

Repeated examinations of the stools revealed a trace of blood on one occasion. Amoebae were not found although they had been reported on one previous examination.

This patient varied from the preceding one in that her appetite was poor. At one time she had gained considerable weight while on forced feedings in a sanatorium under the care of a neurologist. Because of the peculiar facies, she was given the same extract of the anterior lobe of the pituitary as the first patient; slight glossitis, which was present, immediately improved. By February, 1940, she had gained 13 pounds in weight; her strength was sufficient to take care of a household, a four-room apartment, and normal social activities. She still has some diarrhea, especially when she is under a nervous strain. The menstrual periods have become more regular, normal, and painless.

DISCUSSION

With the exception of the loss of hair the patient described as L. W. answers well the description of Simmonds' disease. He had extreme asthenia, was apathetic, had lost his libido, and had lost weight persistently. He had the low basal metabolic rate described in pituitary cachexia, and because of this a myxedema was considered. He showed no reaction to thyroid therapy. He was given insulin in the hopes of increasing a utilization of sugar. With a diagnosis of an avitaminosis he was given for a long period a high caloric diet with vitamins added in the form of yeast and intramuscular injections of a liver extract. Because of the acute glossitis he was given sufficient quantities of nicotinic acid to cause amelioration of such symptoms. It is interesting to note that the glossitis did not disappear until he had been given the extract of the anterior lobe of the pituitary gland.

In the second patient, the sole criteria for diagnosis have been the peculiar facial expression, the loss of weight, the extreme asthenia, and the unstable nervous system. It is true in her case that anorexia nervosa must be considered. Anorexia nervosa is the most common condition confused with Simmonds' disease. In this instance the diarrhea is a symptom not usually present in anorexia nervosa.

She had received considerable care during the preceding five years, when she had been treated by one physician for an amoebic dysentery and by others for a neurosis.

Within two weeks after the institution of the intramuscular injections of extract of the anterior lobe of the pituitary, there were a marked change in her emotional stability and a definite gain in weight and strength. This improvement has continued up to the present time, with a total gain of 13 pounds.

It is true that she still has spells of diarrhea, which consist of 3 to 4 stools, usually in the morning. The total quantity of feces expelled by such diarrheal attacks is considerably less than a pint. These attacks occur as the result of situations that upset her mentally, and are especially likely to occur just before attendance at some social function.

CONCLUSIONS

1. Two persons with cachexia are reported greatly improved or fully recovered following the administration of extract of the anterior lobe of the pituitary gland.

2. It is believed that these two patients represent atypical instances of Simmonds' disease or dysfunction of the anterior lobe of the pituitary.

3. It is possible that the second patient represents a case of anorexia nervosa, although the response to the glandular therapy makes it appear proper to include her in these case reports.

4. The prompt recovery of the glossitis in both patients, and of the reddened rectal mucosa in the first patient, obviously suggests a relationship between the function of the anterior lobe of the pituitary gland and the utilization of the vitamin B complex. Evidence in support of this suggestion will be presented in a forthcoming paper on pellagra.

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LABORATORY METHODS

AN APPARATUS FOR CONSTANT INTRAVASCULAR INJECTION OF LIQUIDS*

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THE apparatus here described is characterized by its simplicity of construction and its high degree of efficiency. It was designed for use in experimental investigations in which it is desired to introduce various liquids into the blood stream (vein or artery) at a constant rate of flow.

Repeated tests of the apparatus, performed by causing it to deliver water into microburettes, have demonstrated that both the rate of flow and the volume of water delivered per unit of time remain constant during observations conducted over a period of one to one and one-half hours, i.e., the period required to completely empty the syringes used when filled to capacity.

The essential construction of the device includes a constant speed motor which drives a nuggled disk by a hard rubber friction drive wheel that is attached to the revolving shaft of the motor. The disk operates a pinion and gear reduction to a long screw which is thus kept in a constant forward motion and propels the piston of one or more syringes. An adjustment is provided on the screw to automatically stop the motor when the pistons have reached the end of the barrel of the syringe.

The only adjustment or replacement that may be required, after long continued use of the apparatus, is that due to slight wearing down of the circumference of the rubber friction drive wheel. When necessary the wheel can be replaced with a new one in a few minutes. It is advisable also to clean the nuggled surface of the disk occasionally to prevent accumulation of dirt which may sometimes cause the drive wheel to slip in spots.

A synchronous motor is essential for constant speed. Such a motor, however, has much less power than a shaded pole induction motor of the same size. In most constant injection experiments, a physiologic range of constancy can be obtained with the induction motor. The motor variation under a continuous load is insignificant, sometimes not more than about 0.2 per cent. We have found the shaded pole induction motor† entirely satisfactory in our experimental work involving constant injections of aqueous or physiologic saline

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†Obtained from the Merkle-Korff Gear Co., Chicago, Ill.

solutions. The apparatus is illustrated in Fig. 1. The following is a detailed description of the apparatus, its construction and operation.

The motor *(1)* is set on two rods *(2)* so that its position can be adjusted in relation to the nuggled disk *(7)* by manipulating the handle that is attached to a screw *(3)* which moves the motor forward or backward on the supporting rods. A line or arrow on the side of the motor serves as an indicator that can be adjusted, by moving the motor, to various positions on a scale *(4)*, placed alongside and just below the motor. This enables the observer to determine the speed of propulsion of the piston *(17)* in the syringe *(18)* and the corresponding rate of delivery of its contents after a preliminary determination is made for the syringe employed. Speed of propulsion increases as the drive wheel is moved from circumference toward the center of the nuggled disk, and vice versa.

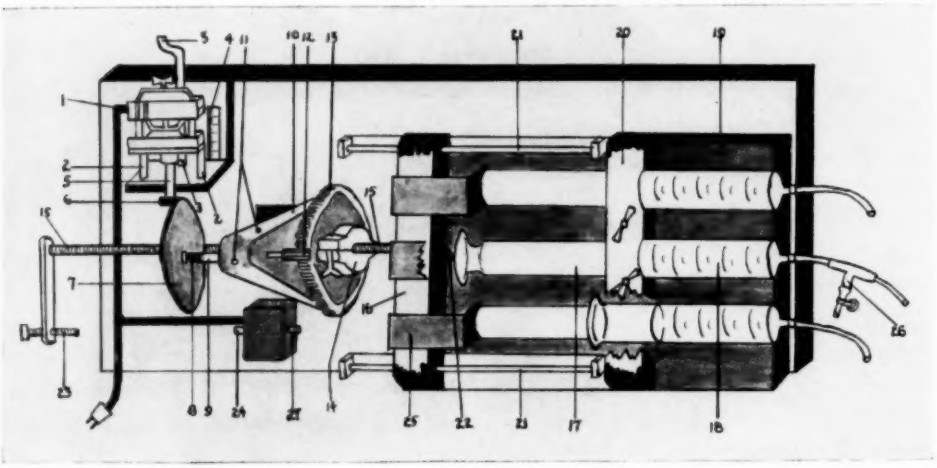

Fig. 1.

The motor rotates a shaft *(5)* at 50 r.p.m. At the end of the shaft is attached a hard rubber friction drive wheel *(6)*, 2.5 cm. in diameter and 4 mm. wide at the circumference, which rests against a nuggled disk *(7)*. The disk, 9 cm. in diameter, is held firmly in contact with the drive wheel by the aid of a spring *(8)* which is coiled around a portion of the drive shaft *(9)* that protrudes from the support *(10)*. When the motor is moved forward or backward, pressure on the disk toward the support permits the drive wheel to be adjusted in the desired position without friction against the disk; releasing the disk causes it again to contact firmly with the drive wheel. Lubrication of the drive shaft is provided for by oil holes *(11)* in the support.

The shaft operates a pinion *(12)* which drives a geared wheel *(13)* to which is attached a split clamp *(14)*. Within this clamp are the threads (see Fig. 2) that propel a long (30 cm.) threaded rod or screw *(15)* as the clamp rotates with the wheel to which it is fastened. The long screw passes through the support and the geared wheel without contact, and it is propelled forward without rotating.

At the forward end the screw is fastened to a wooden block *(16)* which propels the piston *(17)* of one or more syringes *(18)* that lie in grooves of a wooden support *(19)* where they are held firmly by a grooved wooden clamp *(20)* that constitutes the upper part of the support. The block is moved along on two lubricated rod supports *(21)* and to it are fastened rubber knobs *(22)* which press against the heads of the pistons, propelling them in the cylinders of the syringes.

A set screw *(23)* is adjusted to the outer end of the long screw so that it contacts the "off" button on the switch *(24)*, when the syringe is empty, and automatically stops the motor.

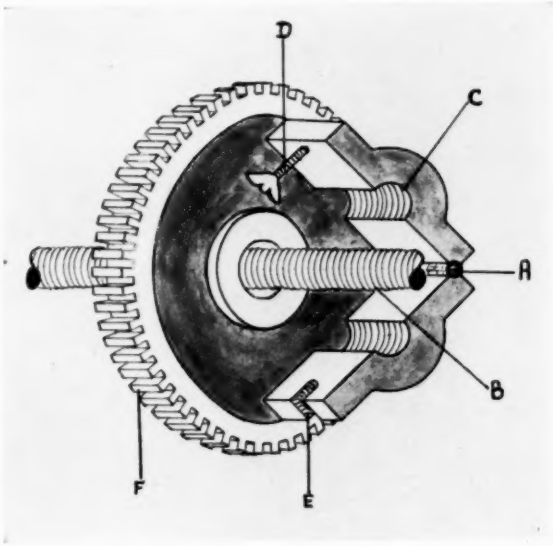

Fig. 2.

Fig. 2 illustrates the construction and operation of the split clamp *(14)* which propels the long screw *(15)* that controls the movement of the piston in the syringe. If it is desired to continue an injection beyond the period required to empty the syringe (or syringes), refilling the syringe requires only a few seconds. The split clamp is opened by loosening the nut on the threaded bolt *(D)* which fits into a groove *(E)* on the opposite half of the clamp. This permits the clamp to open at the hinge *(A)*, releasing the threads *(B)* of the long propelling screw (*15*, Fig. 1) which is driven by the threads (*C*) in the split clamp as it revolves with the geared wheel *(F)* to which it is attached.

To refill the syringes, the block which propels the pistons of the syringes is pushed back so that the screw passes unrestricted through the split clamp, geared wheel, and support, to the original starting position. A metal saddle *(25)*, which rides on top of the block, is notched so that it grasps the head of the piston of the syringe. As the block is displaced backwards the saddle draws back the piston and the fluid to be injected is drawn into the cylinder of the syringe through a side tube *(26)* provided for this purpose. While this is done, the clamp is removed from the rubber tubing on the glass side tube (*26*, Fig. 1) and

placed on the tubing attached to the syringe. The clamp is replaced on the side tube before starting the motor again for continuing the injection. The split clamp is again closed around the propelling screw, and the motor started by pressing the "on" button *(27)* of the switch.

The apparatus at present employed in this laboratory is provided with three 50 c.c. syringes. Using one syringe, with the drive wheel adjusted at the outer margin of the nuggled disk, it delivers 0.7 c.c. per minute; if the drive wheel is moved toward the center of the disk, the speed is increased until 2.5 c.c. per minute is delivered when the drive wheel is close to the center of the disk. Thus the range is from 0.7 c.c. (one syringe) to 7.5 c.c. (three syringes) per minute. Of course, it is possible to increase the number of syringes employed, permitting the performance of a number of experiments with a corresponding number of animals at the same time.

The speed of propulsion can be reduced by employing drive wheels of smaller diameters and by using disks of greater diameters. Furthermore, the quantity of liquid delivered can be reduced by employing syringes with cylinders of smaller diameters. Thus, by a combination of these factors, which can easily be introduced into the construction of the apparatus, it is possible, quantitatively, to vary greatly the speed and volume in the delivery of the fluid injected.

The author is indebted to Mr. Ben Supovitz for drawing the illustrations.

THE PHOTOELECTRIC MICRODETERMINATION OF NITROGENOUS CONSTITUENTS OF BLOOD AND URINE BY DIRECT NESSLERIZATION*

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WHEN Nessler's solution is added to a dilute solution of ammonium salts, an amber-red color is produced which is proportional to the concentration of ammonia in the solution and which can, therefore, be used for the colorimetric estimation of the ammonia. However, if the concentration of ammonia is too great, the colored substance is precipitated. Even in dilute solutions precipitation or clouding occurs on standing; but before it occurs, the color becomes progressively more intense. Many factors other than time also influence the intensity of the color or the speed of cloud formation. Among these are the following: the proportion of Nessler's solution used to the final volume of the solution, the concentration of salts, such as sodium sulfate; the pH of the final solution; the presence of substances which form precipitates with mercury ions; traces of organic solvents, particularly acetone; substances other than ammonia which produce a color with Nessler's solution. To these factors should be added

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the constant presence in almost all laboratory reagents as well as on laboratory glassware, filter paper, and other equipment, of traces of ammonium salts or of substances producing ammonia on ashing, or of substances influencing the color produced by Nessler's solution.

Accurate determination of ammonia nitrogen by nesslerization must take all these factors into consideration. The technique employed must minimize these sources of error and make them constant both in the standard and in the unknown solutions. One of the methods of minimizing the interfering factors is to aerate or distill the ammonia formed into an acid solution and then to nesslerize this solution. However, if such procedures are used, the determinations are then more conveniently and accurately made by volumetric rather than by colorimetric methods. It is to avoid aeration or distillation, which is usually a macroprocedure and which is time-consuming and requires elaborate apparatus, that most biological laboratories make use of methods involving direct nesslerization. Many of these methods have not only the usual subjective errors of ordinary colorimetry but also the much more significant errors produced by failure to consider the many factors which influence the color produced by Nessler's solution.

The photoelectric colorimeter, when used with the proper light filter and with precautions of producing constant conditions, permits much more accurate as well as more rapid determinations than do instruments which require subjective color matching. Furthermore, since pale colors are read with as great accuracy as more intense ones, the photoelectric instruments make possible an ultramicrotechnique unthought of before in routine laboratories. However, the unmodified adaptation of the crude methods of ordinary colorimetry to the photoelectric colorimeter would hardly be worth while; for, though the subjective errors would be removed, the gross technical errors would be untouched. Besides this, other errors are introduced because of a fundamental difference in the two techniques. In color matching constancy of conditions is not so important since unknown and standard are made up simultaneously, whereas in photoelectric methods standard curves are made up at one time while the unknown may be analyzed months or years later with different or aged reagents, and at a possibly different temperature. The adaptation, then, of colorimetric methods to the photoelectric colorimeter requires careful study of all these sources of error. If, however, these sources are eliminated or controlled, the resulting methods are refined and rapid, and have the accuracy of the best volumetric methods.

The following procedures for the determination of the nitrogenous constituents of blood and urine by direct nesslerization were designed to offer such advantages. They are based on the micro-Kjeldahl technique of Koch and McMeekin.[1] The amber solutions obtained have absorption bands between 400 and 450 mμ and are, therefore, best analyzed with a blue filter (Ceneo filter No. 1, which, with an incandescent source of light, has an effective maximum transmission at 430 mμ). About 0.025 mg. of ammonia nitrogen is used for the final color formation, which, with 3 c.c. of Nessler's solution in a final volume of 20 c.c., gives readings around 50 on the microgalvanometer when water is set at 100. The amount of sulfuric acid chosen is the least possible for convenient ashing, so as to minimize the concentration of the sodium sulfate in the final solution.

In the determination of nonprotein nitrogen a trichloracetic acid filtrate of blood has been chosen for analysis rather than the usual Folin-Wu filtrate, because the latter solution contains substances which when ashed may produce clouding on addition of Nessler's solution. It will be noticed that all standard curves are made by determination of standard solutions subjected to the same procedures as the unknown solutions, so as to include the blank in the curve. That the blank values are different for different procedures can be recognized from Fig. 1, where the readings for the same amount of nitrogen are different in the three procedures.

METHOD

Reagents and Apparatus.—1. 2.4 N H_2SO_4. Into a 1,000 c.c. beaker, containing about 600 c.c. of water, add slowly with stirring 67.2 c.c. of concentrated sulfuric acid. Cool and make up to a liter in a volumetric flask. (Keep this, as well as other reagents, protected from ammonia vapor.)

2. 1.2 N H_2SO_4.

3. 30 per cent H_2O_2, C.P.

4. 5 per cent trichloracetic acid.

5. 22.5 per cent Na_2SO_4. This solution is made up from anhydrous sodium sulfate with water at 37° C. and kept in an incubator at that temperature.

6. Nessler's solutions (Koch's modification[1]). Several liters should be made up at a time and kept in a tall bottle. A small amount of precipitate settles to the bottom on standing. It is futile to filter this off, as it will form again. The quantity needed for the day should be pipetted off from the top. The solution will keep indefinitely.

7. Pyrex 25 c.c. test tubes, marked for 20 c.c. with a file. These should be washed with distilled water just before using. Other test tubes, which must be dry at the time of use, should be kept stoppered when not in use.

The Determination of Nonprotein Nitrogen.—One-half cubic centimeter of blood or serum is mixed in a small dry test tube (15 by 125 mm.) with 2.0 c.c. of water and then treated with 2.5 c.c. of 5 per cent trichloracetic acid. (When urine containing protein is to be similarly analyzed, it should first be diluted so that the nonprotein nitrogen concentration is not more than 100 mg. per 100 c.c. The diluted solution is then treated as previously.) After being thoroughly stirred and allowed to stand for several minutes, the mixture is filtered on a folded 7 cm. filter paper, which has been handled as little as possible. (Whatman No. 40 filter paper has been found satisfactory.) The first few drops of filtrate are caught in the original tube and refiltered. Exactly 0.5 c.c. of this filtrate is then transferred to a pyrex test tube marked for 20 c.c. To it is added 0.5 c.c. of 2.4 N sulfuric acid. The tube is placed in a bath of saturated calcium chloride and heated for several minutes at about 130° C. until charring begins. (This preliminary step may be omitted, but it serves to remove the water and chloroform, formed from the decomposition of the trichloracetic acid, without the annoying spattering that occurs when the tube is heated immediately over a free flame.) The tube is then removed from the bath and heated with constant agitation over a free flame until fumes of sulfur trioxide fill the tube. After about forty-five seconds of cooling, 1 drop of 30 per cent hydrogen

peroxide is added from a capillary pipette directly to the sulfuric acid. Decolorization is immediate, but the tube is heated again with agitation for about two minutes, at which time the ashing is complete. The cooled solution is diluted to about 15 c.c. and treated with 3 c.c. of Nessler's solution from a pipette reserved for that purpose. It is then made up to 20 c.c., thoroughly mixed with the aid of clean rubber stopper, and read in the photoelectric colorimeter within two minutes.* When several determinations are made at the same time, it is more accurate to make the reading after each nesslerization rather than to nesslerize all tubes at one time. However, readings at five minutes are usually not more than 0.3 lower than those at two minutes.

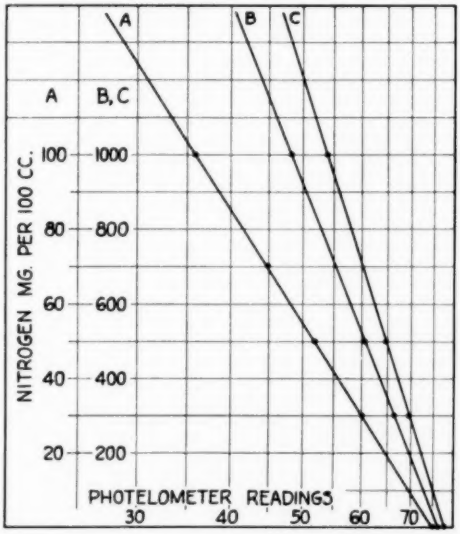

Fig. 1.—The relationship between photelometer readings and concentration of nonprotein nitrogen (Curve A), concentration albumin nitrogen plus nonprotein nitrogen (Curve B), and total nitrogen (Curve C). In Curve A, 0.02 mg. nitrogen reads 55.5; in curve B, it reads 55.8; and in Curve C, it reads 58.0.

Calculation is made by reference to a curve or table of values obtained from standard solutions. For this purpose 4.7166 Gm. of purest ammonium sulfate are dissolved in 100 c.c. of water. This is the stock solution for all the nitrogen analyses described here. It contains 10 mg. nitrogen per cubic centimeter. If all the curves required are made within a day or two, it is unnecessary to add any preservative to this solution, which thus eliminates a possible source of error. Of this stock solution, 3, 5, and 7 c.c. are diluted to 100 c.c. in volumetric flasks, making solutions containing 30, 50, and 70 mg. nitrogen per 100 c.c., respectively. Analyses of these solutions, as well as of a blank of water alone, are carried out exactly as for blood, including the filtration and ashing. With the proper light filter it will be found that the points, when plotted on semi-logarithmic paper as in Fig. 1, fall on a straight line.

The Determination of Total Nitrogen in Serum and Urine.—To 20 c.c. of 2.4 N sulfuric acid in a test tube is added 0.1 c.c. of serum or urine measured in

*The Cenco-Sheard-Sanford photelometer,[3] manufactured by the Central Scientific Co., was used in all these experiments.

a pipette calibrated "to contain." The pipette is washed out several times with the sulfuric acid in the tube. Exactly 0.5 c.c. of the well-mixed solution is transferred to a pyrex test tube marked for 20 c.c. and heated for several minutes in a boiling water bath. (This preliminary step may be omitted, but it serves to hydrolyze the protein and thus prevents foaming.) The tube is then heated in a free flame with rapid agitation until charring occurs and sulfur trioxide vapors fill the tube. After forty-five seconds of cooling, a drop of 30 per cent hydrogen peroxide is added; this decolorizes the solution. The heating is then continued gently for two minutes. The tube is cooled under the tap, and water is added to about 15 c.c., followed by 3 c.c. of Nessler's solution. The solution is made up to 20 c.c., mixed thoroughly, and read in the colorimeter within two minutes. The complete determination can be made in less than ten minutes.

Calculation is made by reference to a table or curve of values obtained from standard solutions. The stock solution, containing 10 mg. nitrogen per cubic centimeter, is diluted with water to give solutions containing 5 and 3 mg. nitrogen per cubic centimeter. These solutions, as well as water alone and the stock solution, are analyzed as above by mixing 0.1 c.c. of each with 20 c.c. of 2.4 N sulfuric acid, using the same pipette calibrated "to contain." One-half cubic centimeter is ashed, treated with hydrogen peroxide, diluted, treated with 3 c.c. of Nessler's, and made up to 20 c.c. The points should fall on a straight line when plotted on semilogarithmic paper, even when extended up to 14 mg. nitrogen per cubic centimeter (see Fig. 1). The total protein concentration is calculated from the total nitrogen in the usual way by subtracting the non-protein nitrogen concentration and multiplying by 6.25. In the analysis of concentrated urines, if the reading obtained shows a total concentration higher than 14 mg. per c.c., it is better to dilute the urine with an equal quantity of water and repeat the determination.

The Determination of Albumin and Globulin in Serum (After Howe[2]*).*—To 3 c.c. of 22.5 per cent sodium sulfate in a small test tube kept in a beaker of water at 37° C., is added 0.1 c.c. of serum from a pipette calibrated "to contain." The pipette is washed out several times with the sodium sulfate solution. The contents of the tube are mixed by inversion, using a clean rubber stopper. The tube is replaced in the beaker of water which is now kept in an incubator at 37° C. for two hours. The precipitated globulin is now filtered off on a folded 7 cm. filter paper (Whatman No. 42). The first few drops of the filtrate are caught in the original tube and refiltered. The filtrate should be perfectly clear. If it is not, a finer grade of filter paper must be used. It is not necessary to filter off more than a few drops of liquid. Two-tenths cubic centimeter of this filtrate are transferred with a pipette calibrated "to contain" into a tube containing exactly 2 c.c. of 1.2 N sulfuric acid. The pipette is washed out several times with the sulfuric acid solution. Of this mixture exactly 1 c.c. is transferred to a pyrex test tube marked for 20 c.c. and ashed as in the previous procedures with the aid of a drop of 30 per cent hydrogen peroxide. It will be found that the final clear solution solidifies on cooling because of the considerable amount of sodium sulfate present. About 15 c.c. of water are added with stirring to dissolve these crystals. The well-mixed solution is treated with 3 c.c. of Nessler's reagent diluted to 20 c.c., mixed, and read in the colorimeter within two minutes.

Calculation is made by reference to a table or curve of values obtained from standard solutions. The stock solution containing 10 mg. nitrogen per cubic centimeter, and dilutions containing 5 and 3 mg. per cubic centimeter, and water alone are all analyzed in the same way as serum, using the same pipettes and filtering with the same type of filter paper. The points should fall on a straight line when plotted on semilogarithmic paper (see Fig. 1). The values obtained represent albumin nitrogen plus nonprotein nitrogen. The albumin concentration is obtained by subtracting the nonprotein nitrogen concentration and multiplying by 6.25. Subtracting the albumin concentration from the total protein concentration gives the globulin concentration.

COMMENT

The methods outlined involve no new principles but are offered as a technique which permits rapid but accurate determinations with minute amounts of material. They serve, too, to call attention to remediable errors in previous methods of direct nesslerization by insisting on constancy of conditions of the determinations and by inclusion of the blank in the determination of the standard solutions.

The substitution of ordinary test tubes for the large cumbersome 60 c.c. micro-Kjeldahl tubes used in most laboratories should be welcomed. However, for those who insist on using these larger tubes and who believe that a slightly less microprocedure would be more accurate, it is recommended that the same proportions of reagents and fluids to be analyzed as outlined here be used. If the amount taken for the final ashing is 2.5 times the amount suggested here and if the ashed material is made up to 50 c.c. with 7.5 c.c. of Nessler's solution, curves similar to those in Fig. 1 will be obtained with probably less relative blank from the filter paper.

The accuracy of these micromethods is attested by comparison with analyses made on the same material by macro-Kjeldahl procedures with titration of the distilled ammonia (see Table I).

TABLE I

ACCURACY OF DETERMINATION OF NITROGENOUS CONSTITUENTS

The results are expressed in mg. per 100 c.c.

SAMPLE	N.P.N. METHOD		TOTAL NITROGEN METHOD		ALBUMIN NITROGEN METHOD	
	AUTHORS'	MACRO-KJELDAHL	AUTHORS'	MACRO-KJELDAHL	AUTHORS'	MACRO-KJELDAHL
Blood	32.0	31.4				
Blood	38.8	38.1				
Serum	27.0	26.5	1,040	1,010		
Serum	24.5	23.7	1,200	1,205	760	780
Serum	26.5	27.5	1,180	1,162	775	800
Serum	23.0	23.2	850	843	432	425
Urine			1,350	1,310		
Urine	750	738				

SUMMARY

A technique is offered for the microdetermination of the nitrogenous constituents of blood and urine by direct nesslerization, using the photoelectric colorimeter. It is rapid, convenient, and accurate.

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A MICROPHOTOELECTRIC METHOD FOR UREA DETERMINATION IN BLOOD AND URINE BY DIRECT NESSLERIZATION*

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MOST modern methods for the determination of urea in blood and urine involve the conversion of urea into ammonium carbonate by aid of the enzyme urease. The latter is usually obtained as a crude extract of jack bean meal. The ammonium carbonate formed has been analyzed in the more precise procedures by measuring the extractable carbon dioxide from the acidified solution, as in the manometric method of Van Slyke,[1] or by aerating the ammonia from the alkalinized solution into a standard acid solution and titrating the residual acid.[2]

Neither of these two methods is widely used in biological laboratories where a large number of routine determinations of urea must be made daily. The manometric method requires special apparatus and a high degree of technical proficiency. The aeration method also requires special apparatus and is time-consuming. In this method 3 c.c. of blood must be used for each determination. Besides, if the air current is not controlled, there may be large errors. For these reasons, many biological laboratories have resorted to methods involving direct nesslerization. A number of these methods have appeared, the first and simplest of which was probably that of Karr,[3] who added urease to a Folin-Wu filtrate and nesslerized without attempting to remove the urease. Gruskin[4] was apparently the first of a series of investigators to advocate the addition of urease directly to blood and serum, and the subsequent removal of both the blood proteins and urease by a standard deproteinization technique. The filtrate obtained supposedly gave clear amber solutions with Nessler's reagent, the color of which was proportional to the amount of urea in the fluid.

When we attempted to adapt a method of direct nesslerization to the use of the photoelectric colorimeter, we were immediately impressed with the lack of accuracy of most of the procedures. There were two obvious sources of error which could be ascertained easily with the photoelectric colorimeter. The first was the difficulty in obtaining absolutely clear solutions on the addition of Nessler's solution, even when the urease was removed with the blood proteins. Both the blood and the urease solutions contained soluble substances which produced clouding on addition of Nessler's solution and which were not easily

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removed by any of the standard deproteinization methods. The second source of error was the variable amount of color-producing substances in the filtrates other than ammonia that had been formed from urea. If this source of error were constant, it might not produce much difficulty, for its value could easily be determined with the photoelectric colorimeter. But it was not constant and it was often quite large, representing from 3 to 10 mg. of urea nitrogen per 100 c.c.

A number of methods of deproteinization were investigated in an effort to obtain a filtrate from the blood-urease mixture which would produce clear solutions and which would contain a minimum of nonammonia color-producing substances. Folin-Wu filtrates were found unsatisfactory, as were those made with trichloracetic acid and with toluene sulfonic acid. All these caused clouding. The use of cadmium sulfate and sodium hydroxide, recently recommended by Fujita and Iwatake[5] for the determination of true glucose because it yielded filtrates singularly free of nonsugar reducing substances, also proved disappointing. Clouding was obtained both in filtrates made in the cold and in those after heating. The Somogyi[6] procedure, using zinc sulfate and sodium hydroxide suggested by Peters and Van Slyke,[7] furnished filtrates that gave clear solutions with Nessler's reagent, but the blank obtained was large and variable. Besides, the filtration was annoyingly slow. However, when the mixtures were heated in a boiling water bath for two minutes, the subsequent filtration was rapid and yielded a filtrate that remained clear on the addition of Nessler's reagent and produced only a small and relatively constant blank.

Since in all photoelectric procedures, curves of values from standard solutions must be obtained by a technique that is constantly reproducible, it was found necessary to use a standard urease solution which was freshly prepared for each set of determinations in order to have the same blank from the urease. Obviously, the most exact method is to weigh out accurately the required amount of dry jack bean extract for each urease solution. This tedious procedure could be avoided by using urease tablets of approximately constant weight. Tablets of urease made by the Arlington Chemical Co. were found to be uniform and firm.

The colorimetric determination of urea involves the consideration of other sources of error inherent in all nesslerization procedures. These have been discussed in another paper.[8] It is probably of value to emphasize that inasmuch as no ashing is required here which would destroy any traces of the organic solvents used in the cleansing of pipettes, particular care must be employed to avoid their presence. Acetone is particularly harmful. The slightest trace of acetone has been found to produce sufficient clouding to cause large errors in this micromethod. For this reason, we no longer use acetone in cleansing and drying pipettes, but have returned to the use of alcohol and C.P. ether. There is an appreciable blank from filter paper. It is worth while, therefore, to use an ashless filter paper of uniform quality, such as Whatman No. 40.

METHOD

Reagents.—1. Urease solution. A tablet of Arlco-urease is ground in a clean dry mortar with exactly 10 c.c. of 0.05 disodium acid phosphate. The turbid

solution is transferred to a small tube reserved for this purpose. The solution must be used within two or three hours.

2. Zinc sulfate, 10 per cent. A trace of precipitate may form on standing. It settles to the bottom and may be ignored.

3. Sodium hydroxide, 0.50 N.

4. Nessler's Solution (Koch-McMeekin[9]).

Procedure.—Into a small dry test tube (15 by 125 mm.) 0.2 c.c. of blood or serum or urine, diluted 25 times, is transferred with a Van Slyke-Neill pipette. Then 0.2 c.c. of urease solution is added. The tube is rotated to assure thorough mixing, then stoppered with a clean rubber stopper, and placed in a water bath at 45° C. for fifteen minutes. Four cubic centimeters of water are added, followed by 0.3 c.c. zinc sulfate reagent, and 0.3 c.c. sodium hydroxide, with agitation of the contents of the tube after the addition of each reagent. The tube is placed in a beaker containing an amount of boiling water just sufficient to reach the level of the contents of the tube. After two minutes in the boiling water bath (which should not appreciably reduce the volume of liquid since the upper part of the tube acts as a condenser), the tube is cooled to room temperature and the contents filtered through a folded 7 cm. filter paper (Whatman No. 40). The first few drops are caught in the original tube and refiltered. Exactly 2 c.c. of the filtrate are transferred to a 25 c.c. test tube marked for 20 c.c. with a file. About 15 c.c. water are added, then 2 c.c. of Nessler's solution from a pipette reserved for that purpose. The solution is made up to 20 c.c., mixed by inversion with the aid of a clean rubber stopper, and read in the photoelectric colorimeter* within two minutes. A blue filter, with a maximum transmission at 430 mμ (Cenco No. 1), should be used. A blank determination can be made by treating the blood or diluted urine as in the determination of urea by adding the zinc sulfate and sodium hydroxide before the urease and heating immediately for two minutes without any previous digestion. For blood we have found the blank to be of the order of 2 mg. urea nitrogen per 100 c.c., which can be assumed for all determinations. However, the urine blank includes the value of the preformed ammonia and must, therefore, be determined in each case. This determination, too, might theoretically be avoided if a permutit-treated urine is used for the determination, but since even such liquids may still show appreciable and variable blanks, it is better to avoid the use of permutit and to determine the blank which includes the preformed ammonia.

Calculations are made by reference to a table or curve of values obtained by analyses of standard solution of urea. A stock solution of urea, containing 80 mg. urea nitrogen per 100 c.c., is made up by dissolving 1.7144 Gm. of pure, dry urea in water and making up to a liter in a volumetric flask. A drop of toluene is added as a preservative. Exactly 5, 10, and 15 c.c. of the stock solution of urea are diluted to 50 c.c. in volumetric flasks, making solutions containing 8, 16, and 24 mg. urea nitrogen per 100 c.c., respectively. These and water alone are analyzed exactly as in the procedure for blood, using 0.2 c.c. of the

*The Cenco-Sheard-Sanford photelometer, manufactured by the Central Scientific Co., was used in all these experiments.

standard solution and 0.2 c.c. of urease solution, and carrying out the digestion, deproteinization, heating, and filtering as previously. Two cubic centimeters of the final filtrate are made up with water and 2 c.c. of Nessler's solution to 20 c.c. and read in the photoelectric colorimeter within two minutes. The curve obtained when plotted on semilogarithmic paper is a straight line (Fig. 1). In the determination of blood of high urea concentration, it is more accurate to analyze the diluted blood than to use less of the final filtrate for nesslerization, since, in the latter case, there will be less blank.

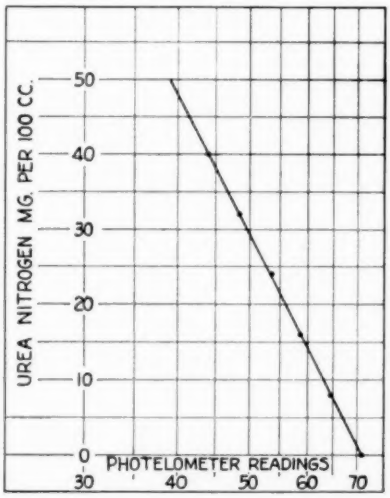

Fig. 1.—The relationship between photelometer readings and urea nitrogen concentration.

The standard curve should be repeated with several urease solutions to check on the uniformity of the tablets, for a good portion of the blank comes from the urease. If the tablets are not uniform, it is suggested that a glycerol solution of urease, as recommended by Wren,[10] but containing no acetone, be used. Under any circumstances, it must be recognized that unless the blank of the urease solution is duplicable, photoelectric microanalysis of urea by direct nesslerization is not reliable.

Table I compares the analysis of urea by the proposed method with that by the aeration method of Van Slyke and Cullen.[2] There is close agreement.

TABLE I

ACCURACY OF DETERMINATION OF UREA

The results are expressed in mg. per 100 c.c.

SAMPLE	METHOD		ADDED UREA NITROGEN	TOTAL UREA NITROGEN	
	VAN SLYKE AND CULLEN	AUTHORS'		CALCULATED	FOUND
Blood	11.0	11.2	8.0	19.2	19.5
Blood	15.7	15.0	16.0	31.0	31.7
Blood	8.5	8.9	32.0	40.9	40.0
Urine	788	770	400	1,170	1,100
Urine	1,160	1,206	800	2,006	2,040

SUMMARY

A photoelectric method for the microdetermination of urea in blood and urine by direct nesslerization is offered. Duplicability of the values obtained is brought about by a deproteinization technique which gives clear solutions on the addition of Nessler's reagent, with a minimal and constant blank.

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PHOTOMETRIC BLOOD SUGAR DETERMINATION BY THE FOLIN-WU METHOD*

A New Source of Error

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SINCE the application of photometry (optical or electrical) has become so wide as to gradually replace clinical colorimetry, many authors have attempted to adapt previous methods to the new instruments.

One of the most universally accepted methods for blood sugar determination, that of Folin and Wu, has been repeatedly recommended for use with various types of photometers.[1]

Unfortunately, a very important source of error has passed unnoticed: the very marked fading of the color, which takes place immediately after the mixing of the reagents and gradually increases during many hours (Chart 1). It is obvious that, on account of this rapid fading, which seems to have escaped the attention of previous workers,[2] it is absolutely impossible to give any consistent figures for the optical density of the glucose solution (unless all time factors are very rigidly fixed). We have tried to overcome this point by shortening the time necessary to arrive at a final equilibrium, when the fading of the color has practically ceased. We have found that this

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may be obtained by immersing the tubes in a boiling water bath for five minutes after adding the phosphomolybdic reagent (Chart 2). In this way, also, bubbling (which is so troublesome in photometric readings) has been entirely eliminated.

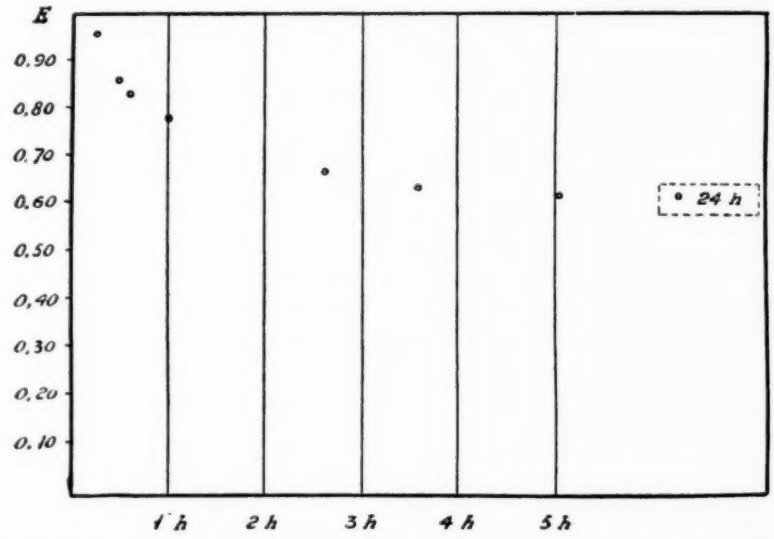

Chart 1.—Fading of color in the course of time, observed in a 0.2 per cent solution of glucose at a depth of 0.5 cm., using a red screen (about 690 $\mu\mu$). It is to be noticed that only after twenty-four hours does the phenomenon become insignificant at room temperature. E = light extinction.

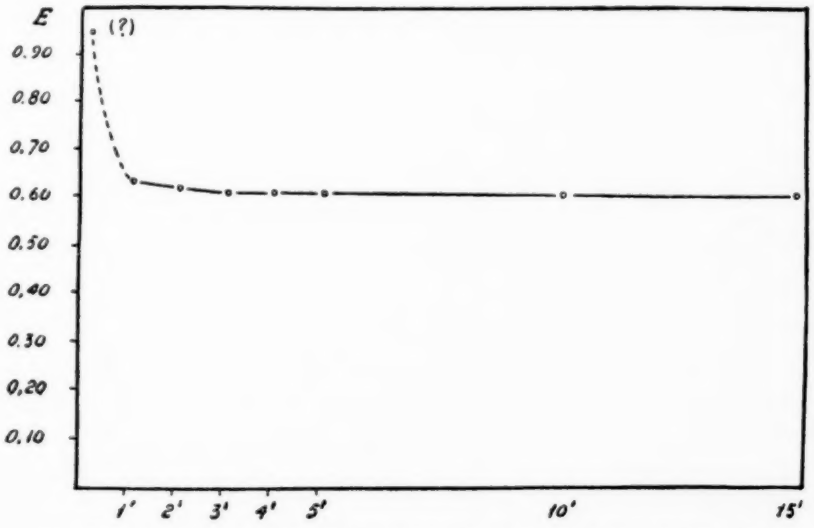

Chart 2.—Loss of color after heating of various duration in a water bath.

Chart 2 shows that after three minutes' heating in a water bath, the color fading has practically reached its final point, so that readings may be made which are easily comparable.

Chart 3 shows the light absorption curve (calculated as extinction) which we have obtained with pure glucose solutions. We obtained a straight line,

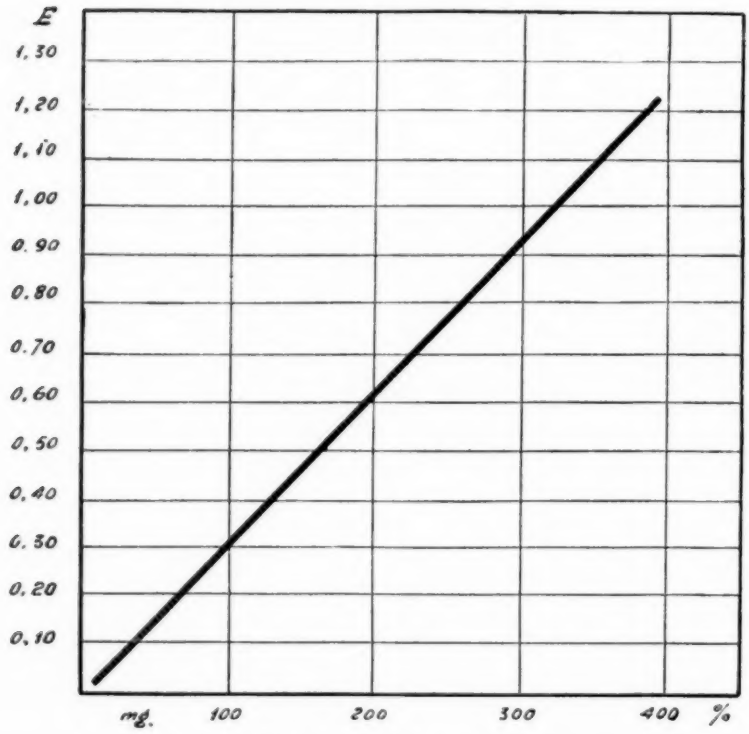

Chart 3.—Color of glucose solutions at various concentrations.

which goes exactly to the zero point. That is to say, there is perfect proportionality between glucose concentration and color production.

Our results are not in accordance with those found in the literature, referring to the alleged nonproportionality of color developed in the Folin-Wu method,[3] but we believe that previous observations are now to be considered as not entirely reliable.

It is obvious that a different chart must be constructed for the use with every optical device in practice, but this is a matter of no real difficulty for any laboratory worker.

SUMMARY

It is demonstrated that the color produced in the Folin-Wu glucose determination fades rapidly at room temperature, thus affecting every photometric reading.

It is suggested to stabilize the color by five minutes' heating in a water bath before reading.

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FURTHER OBSERVATIONS ON THE HEMOLYTIC EFFECTS OF ETHYL AND CAPRYLIC ALCOHOL*

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THE well-known influence of experimental conditions upon the results of hemolysis studies[1] suggested repetition, with slightly modified technique, of some previously reported observations on the hemolytic effects of ethyl and caprylic alcohol.[2] During these studies, it was noted that caprylic alcohol, despite its marked hemolytic properties, was capable under certain conditions of protecting red blood cells against hypotonic laking. Similar results for an extensive series of aliphatic narcotics have been reported by Yoshitomi,[3] and protection against hypotonic hemolysis has been employed by Sollmann[4] as a criterion of astringent action. The present study includes (a) additional observations on the hemolytic properties of ethyl and caprylic alcohol, and (b) observations on the osmotic resistance of red blood cells in the presence of low concentrations of these reagents.

(a) Hemolytic Effects of Water and Alcohols

Sixteen samples of fresh beef blood and 5 of hog blood were used in comparing the hemolytic powers of water, ethyl alcohol, and caprylic alcohol. The reagents were added, not directly to blood as in the previous study,[2] but to 0.9 per cent sodium chloride solution in proportions identical with those previously used. Small amounts of blood were transferred to the resulting test mixtures by means of a stirring rod. The suspensions were thoroughly mixed at intervals of five, fifteen, thirty, forty-five, and sixty minutes, and observations for hemolysis were made at five and at sixty minutes. The criterion of hemolysis was transparency of the mixture. All observations were at room temperature (average 25.3° C.; standard deviation 2.4); comparisons were made at equal temperatures.

The results are summarized in Table I, which shows the dilution of hemolytic agent in salt solution at which hemolysis occurred. Included in the table for comparison are the results of adding reagents directly to blood.[2] Data for beef and hog blood were considered together as previous results failed to reveal a significant difference.

Although the results suggest that, in the cases of water and caprylic alcohol, hemolysis was more readily accomplished by adding the reagents directly to blood, this discrepancy may be due to the fact that transparency was of necessity judged in a slightly different manner for the two series of experiments. The difference in medians, where noted, was only one step in the series of dilutions used. The relative hemolytic powers of water, ethyl alcohol, and caprylic alcohol,

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as previously reported and as indicated by the blood series in Table I were approximately 1, 4, and 200, respectively. In the 0.9 per cent NaCl series, the corresponding values were 1, 8, and 200. It is, therefore, doubtful whether the results express a real difference between the two sets of observations. The previously reported slowness of development of full hemolytic capacity by caprylic alcohol was confirmed as noted in Table I.

TABLE I

HEMOLYSIS BY REAGENT DILUTIONS IN BLOOD AND IN 0.9 PER CENT SODIUM CHLORIDE SOLUTION

	TIME	WATER			ETHYL ALCOHOL			CAPRYLIC ALCOHOL		
		MEDIAN		EXTREMES	MEDIAN		EXTREMES	MEDIAN		EXTREMES
	Min.	*Dil.*	*Per cent*	*Dil.*	*Dil.*	*Per cent*	*Dil.*	*Dil.*	*Per cent*	*Dil.*
Blood	5	1	86.7	1,2	3	86.7	3,4	<10	100.0	
	60	1	86.7	1,2	4	86.7	4,5	200	66.7	100,200
0.9%	5	0.5	71.5	0.5,1	3	100.0		<10	100.0	
NaCl	60	0.5	66.7	0.5,1	4	76.2	3,4	100	54.5	100,400

Notes.—Figures in "dilution" columns represent parts of blood or 0.9 per cent NaCl solution per one part of reagent in the test mixture.

Figures in "per cent" columns represent the percentage of samples having the median value.

(b) Osmotic Resistance in the Presence of Ethyl and Caprylic Alcohol

Resistance to hypotonic laking of red blood cells from 13 samples of beef blood and 5 samples of hog blood was tested by transferring small amounts of blood, as noted above, to sodium chloride solutions having concentrations of 0.25 to 0.60 per cent. In addition to an untreated control series, four series each of test mixtures containing ethyl and caprylic alcohol were prepared for a given sample of blood. The proportions of alcohols used were (reagent: salt solution): 1:1,000, 1:500, 1:250, and 1:125. Readings were made fifteen minutes after the addition of blood to the test mixtures. Osmotic resistance was measured as the midpoint of the zone between complete hemolysis and imperceptible hemolysis. As only large differences were of interest, no attempt was made to measure red blood cell fragility with great accuracy. All observations were made at room temperature (average 26.0° C.; standard deviation 1.8).

Table II is a summary of the results obtained, including the direction of change in osmotic resistance for individual samples. In the ethyl alcohol series, all dilutions showed identical averages and nearly identical distributions. Moreover, the results were so nearly like the control values that no measurable difference could be postulated on average results. Individual samples showed a definite increase of osmotic resistance in 17.6 per cent of the total possible opportunities, the great majority of samples being unchanged regardless of the dilution of ethyl alcohol used. Of the 9 definite changes recorded, 8 were distributed among the 1:500, 1:250, and 1:125 mixtures, accounting for 13.3 to 23.1 per cent of the samples tested.

In the caprylic alcohol series, averages indicate that the resistance to hemolysis was increased. All the caprylic alcohol averages were significantly[5] lower than the control average, although this was true of the distribution of individual

values only for dilutions lower than 1:500.[6] There was also a statistically significant difference between the average osmotic resistance for the 1:1,000 group and that for the 1:250, indicating a progressive increase in resistance to hemolysis between these two classes. The frequency of definite increases in osmotic resistance among individual samples was most impressive at 1:500 and smaller dilutions, where the number of samples showing change was 87.5 to 93.3 per cent of the samples tested. These frequencies were much greater than the corresponding values for the ethyl alcohol series.

TABLE II

OSMOTIC RESISTANCE OF RED BLOOD CELLS IN THE PRESENCE OF ETHYL ALCOHOL AND CAPRYLIC ALCOHOL

(Readings at 15 minutes)

REAGENT	DILUTION	OSMOTIC RESISTANCE				CHANGE IN OSMOTIC RESISTANCE					
		EXTREMES	AVG.	MED.	S.D.	INCREASE		DECREASE		NO CHANGE	
		Per cent NaCl	*Per cent*	*Per cent*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
Control		0.42-0.52	0.47	0.47	0.027						
Ethyl alcohol	1,000	0.40-0.53	0.46	0.46	0.035	1	6.3	0	0.0	15	93.7
	500	0.41-0.52	0.46	0.45	0.034	3	18.8	0	0.0	13	81.2
	250	0.40-0.50	0.46	0.46	0.032	2	13.3	0	0.0	13	86.7
	125	0.40-0.52	0.46	0.46	0.038	3	23.1	0	0.0	10	76.9
Caprylic alcohol	1,000	0.40-0.49	0.44	0.44	0.028	5	29.4	0	0.0	12	70.6
	500	0.38-0.46	0.42	0.41	0.024	14	87.5	0	0.0	2	12.5
	250	0.38-0.45	0.41	0.41	0.019	14	93.3	0	0.0	1	6.7
	125	0.38-0.45	0.40	0.40	0.022	10	90.9	0	0.0	1	9.1

Notes.—Figures in "dilution" column represent parts of sodium chloride solution per one part of reagent in test mixture.

S. D. represents standard deviation from the average.

Only alterations of osmotic resistance in excess of 0.03 per cent NaCl are recorded as changed in this table.

To ascertain the permanency of the changes just outlined, osmotic resistance was measured for 5 samples of blood at hourly intervals over a period of five hours. Alcohol concentrations were the same as those previously mentioned, with omission of the 1:125 dilution. A smaller number of sodium chloride concentrations was employed. No changes in resistance to hypotonic laking were noted in the ethyl alcohol group during the period of observation. Increase in osmotic resistance occurred in a majority of the caprylic alcohol tests up to the end of the second hour. Beyond this point, 1:500 and 1:250 dilutions began to show definite decreases in osmotic resistance which, at the end of the fourth hour, included all samples. In five hours, two out of five 1:1,000 dilutions still showed increased resistance, while the first decrease for this dilution had just appeared. It is apparent, therefore, that the increased resistance to hemolysis caused by the concentrations of caprylic alcohol here employed was limited in duration, the hemolytic powers increasing in prominence with prolonged contact.

SUMMARY

1. The relative hemolytic powers of water, ethyl alcohol, and caprylic alcohol were tested at room temperature over a period of one hour by adding small amounts of blood to various dilutions of hemolytic agent in 0.9 per cent sodium chloride solution. In two-thirds of the samples, water caused hemolysis in dilutions of 2:1. Ethyl alcohol had approximately eight times and caprylic

alcohol about two hundred times the laking power of water. These results were compared with analogous findings for direct additions of hemolytic agents to defibrinated blood. The differences noted were of doubtful significance.

2. Resistance of red blood cells to hypotonic laking was tested at room temperature in the presence of various concentrations of ethyl and caprylic alcohol. Ethyl alcohol caused no changes in five hours at the dilutions employed. Caprylic alcohol in dilutions of 1:500, 1:250, and 1:125 increased the osmotic resistance for at least fifteen minutes in approximately 90 per cent of the samples. Such protective action was diminished in three hours, and only decrease in osmotic resistance occurred after four hours.

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A NUMERICAL SYSTEM USING COLORS FOR MARKING ALBINO RATS AND MICE*

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NUMERICAL systems for designating laboratory animals usually involve tattooing, clipping of ears or toes or both, or leg bands, but white rats and mice are so easily marked by putting spots of color on their coats that this is a very popular method. It is especially so if there are objections to maiming the animals by clipping. Most of the laboratories which employ the coloring method are contented with the use of one or two colors and a standard rotation of marked sites, further differentiation being effected by placing the animals in two or more cages in each of which no two animals bear the same mark or combination of marks. This procedure often is not entirely satisfactory. Sometimes it becomes expedient to place a marked animal in a cage already containing an animal with the same mark, thus necessitating additional marking and a change in the records. Moreover, animals which have escaped from their cages are not easily identified. Finally, these markings require cumbersome entries in the records unless numbers are arbitrarily assigned to represent them.

A system of color marking employed recently in our animal room has proved to combine all of the advantages of a numerical system with those of

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the color technique. The improvement in housing, in record-keeping, and in references to experimental animals used in the past, is obvious. Combinations of colors and sites are used and are arranged in an orderly way so that the number which any combination represents may be recognized immediately without reference to a code. Familiarity with the system is acquired usually before 50 animals have been marked.

METHOD

The nine different locations on the rat stand for the digits from 1 to 9, and the four colors stand for the column in which these digits are to be found. The nine digits are represented conveniently by the following nine sites:

1. Left ear
2. Right ear
3. Left shoulder
4. Back of neck
5. Right shoulder
6. Middle of back
7. Left hip
8. Lower back
9. Right hip
0. No mark at all

Although the snout is a desirable location on account of its visibility, it is not given a number because the marks placed there vanish too quickly. On the sites listed the colors last for about three weeks and are renewed, as any dye markings must be, when they are about to become indistinct. The four colors, yellow, red, green, and purple, show whether the digits belong in the ones, tens, hundreds, or thousands column, respectively. This order is chosen according to the permanence of the stains on the coats of the animals; the numbers in the ones and tens column are usually the most significant and are, therefore, assigned the most lasting colors. The practical application of the system is shown best by the examples in Table I. Obviously, a number in which the same digit occurs more than once (55, 1232, 6686, etc.) cannot be represented, for that would entail the application of two colors to the same site. It is for this reason that not all of the 9999 numbers are available. The same digit appears two or more times in 4491 numbers, thus leaving 5508 which are suitable for use.

TABLE I

MARK	PURPLE (*n*– – –)	GREEN (–*n*– –)	RED (– –*n*–)	YELLOW (– – –*n*)
3				Left shoulder
30			Left shoulder	
300		Left shoulder		
3000	Left shoulder			
34			Left shoulder	Back of neck
43			Back of neck	Left shoulder
403		Back of neck		Left shoulder
430		Back of neck	Left shoulder	
432		Back of neck	Left shoulder	Right ear
4321	Back of neck	Left shoulder	Right ear	Left ear
8967	Lower back	Right hip	Middle of back	Left hip

The colored marks on the animals can be produced by painting on the following solutions: (1) yellow, by a saturated aqueous solution of picric acid; (2) red, by Ziehl's carbolfuchsin solution; (3) green, by a 2 per cent solution

of malachite green in 50 per cent alcohol; and (4) purple, by a saturated alcoholic solution of gentian violet diluted with thirty volumes of 50 per cent alcohol. The purple and green solutions probably may be diluted considerably before they fail to give satisfactory results. One ounce widemouthed bottles stoppered with corks in which camel's-hair brushes have been inserted make convenient containers for these solutions. The bottles are mounted in a row in a wooden block. None of these dyes has been found to be poisonous to the animals.

SUMMARY

A numerical color system for marking white rats and mice is described. With the application of one to four colors on one to four of nine locations on the animal there become available about fifty-five hundred different combinations which may be designated numerically.

METHOD OF SIMULTANEOUS FIXATION AND DECALCIFICATION OF BONE*

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THE method effecting simultaneous fixation and decalcification of bone has been successfully employed in our laboratory for a period of five years. Because of the corrosive sublimate fixation, the resulting preparations are suitable for the application of the majority of the special staining procedures. With this method of decalcification, tissue distortion is minimal, and the staining capacity of the nuclei is not diminished.

Decalcifying-Fixing Fluid:

Alcohol, 95 per cent	50 c.c.
Formaldehyde	40 c.c.
Nitric acid	5 c.c.
Corrosive sublimate	10 Gm.
Trichloracetic acid	30 Gm.
Distilled water	400 c.c.

1. Dissolve corrosive sublimate in 300 c.c. of water *with the aid of heat.* Cool. This is solution No. 1.

2. Dissolve trichloracetic acid in 100 c.c. of water, add nitric acid, alcohol, and formaldehyde. This is solution No. 2.

3. When solution No. 1 is cool, add solution No. 2.

4. The finished stock solution will keep indefinitely in the icebox. At room temperature the mercury tends to precipitate after three or four weeks.

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METHOD

1. Cut sections of fresh bone, not more than 8 to 10 mm. in thickness with a scroll saw.

2. Place sections in decalcifying-fixing solution (at least 100 times as much fluid as tissue) and place in an incubator at 37° C. The entire process of decalcification is carried on at this temperature.

3. Replace the decalcifying-fixing fluid daily until tissue is soft. The degree of decalcification may be tested by palpation or by cutting an extremely thin slice with a sharp knife. The common practice of piercing the tissue with a needle frequently breaks the architecture in the most interesting portion of the section.

4. When the tissue is soft, place in running water for twenty-four hours. (We have found this sufficient; however, 1.5 to 2 per cent ammonia water may be used for twelve hours, followed by running water for twenty-four hours.)

Place tissue in:

80 per cent alcohol for two hours
95 per cent alcohol for six hours
Absolute alcohol for from sixteen to twenty-four hours
Xylol for from five to ten minutes
Oil of cedar (clearing) for twenty-four hours
Xylol (two changes) for thirty minutes
Paraffin 56° to 58° (3 changes) for from four to six hours in all.
Embed.

If the following procedure is employed before staining the intensity of the nuclear stain is increased: (1) Immerse mounted sections (after removal of paraffin) overnight in Bouin's fluid. (2) Wash in running water for from fifteen to thirty minutes.

With certain sections of bone altered by pathologic changes, such as tumor infiltrations, there is danger of maceration when an attempt is made to saw them into small pieces for decalcification. Such tissue may be placed in the decalcifying-fixing fluid for two days or more for partial decalcification. They may then be cut with a sharp knife into sections of suitable size. Decalcification is then continued.

The average piece of bone decalcifies in from three to five days; if it requires longer than seven days, nuclear staining is impaired.

CULTURAL DIAGNOSIS OF TUBERCULOSIS USING BORDET-GENGOU AND LÖWENSTEIN MEDIA*

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CULTURAL diagnosis of tuberculosis has been studied by many investigators with a view of substituting culture for guinea pig inoculation. Although a great deal of work has been done and many excellent media have been devised for this purpose, there is still considerable difference of opinion with regard to the advisability of relying on culture alone.

In a recent review of the literature, Murphy and Duerschner,[1] and Guggenheim and Finkelstein[2] summarized the results obtained by various investigators in comparative studies of culture and guinea pig inoculation. These may be divided into three groups: (1) Equally good results were obtained on culture and guinea pig inoculation. (2) Better results were obtained on culture than on guinea pig inoculation. (3) Better results were obtained on guinea pig inoculation than on culture.

In considering the value of any diagnostic procedure, one must take into consideration not only the accuracy, but also the practicability of the method. Although guinea pig inoculation is undoubtedly an excellent method of detecting small numbers of tubercle bacilli, this method is limited in its application because of the expense involved and also because comparatively few of the small laboratories are equipped to carry animals. Culturing on a suitable medium is relatively inexpensive and can be carried out in any laboratory that has a trained technician. The results obtained on culture by a large number of investigators have been so satisfactory that the adoption of culture as a diagnostic procedure seems to be justified.

The question of an optimum medium for cultural diagnosis is still a matter of opinion. A number of media are available, and good results have been reported with a large number of them.[1, 2] The choice of any particular medium would depend largely on the preference of the individual worker.

One of us (L. M.) reported in 1932[3] the results of a study of the growth of the human and bovine types of tubercle bacilli on various media. We found at that time that of the media tested, the Bordet-Gengou[4] and the Löwenstein[5] egg medium gave the best results. In a second publication[6] in 1934 we reported the results obtained in a comparative study of culturing sputa on the above media and simultaneous inoculation of guinea pigs. Of 233 specimens tested at that time, 18 were positive on the Bordet-Gengou medium, 13 on the

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Löwenstein medium, and 15 on guinea pig inoculation. Based on these findings, we have since then substituted culture on the Bordet-Gengou and Löwenstein media for guinea pig inoculation.

We have cultured since our last publication a variety of specimens which included sputa, tissues, pus, pleural fluids, spinal fluids, urines, and other pathologic materials. Most of the specimens came from patients who had been repeatedly negative on routine smear examinations. Each specimen was examined by smear and only those were cultured that were negative on careful smear examination.

METHOD OF CULTURE

Sputum.—The sputum was poured into a Petri plate and examined for cheesy particles which, if present, were collected by means of a broken-tip pipette or a heavy wire loop, and two thick smears were made. The smears were stained by the Ziehl-Neelsen method, using brilliant green 1:1,000 dilution as a counterstain, and were examined thoroughly for acid-fast bacilli. We found in a previous study[7] that by selecting the material in the manner described the result was the same as concentrating the sputum. If the specimen was negative on smear examination, it was cultured as follows:

The specimen was placed in a sterile 40 c.c. centrifuge tube containing glass beads, and an equal amount of 5 per cent oxalic acid was added (Corper and Uyei method[8]). The tube was stoppered with a sterile solid rubber stopper, and the mixture was incubated at 37° C. for forty-five minutes. During the incubation the mixture was shaken vigorously at frequent intervals. A few drops of bromthymol blue were added after incubation, and the reaction was adjusted to pH 6.8-7.0 with 10 per cent sodium hydroxide. The specimen was then centrifuged at high speed for one hour. The supernatant fluid was decanted, and the sediment was suspended in about 5 c.c. of sterile distilled water, filtered through a thin layer of sterile cotton and inoculated on several plates of the Bordet-Gengou and Löwenstein media. The plates were incubated at 37° C. for two to three days without sealing them to allow the excess moisture to evaporate. They were then sealed with Scotch photographic tape and reincubated for five to six weeks. The cultures were examined microscopically once a week, using a low power microscope.

Pus.—If the pus was very thick, a suspension was made in sterile distilled water; if not, it was used undiluted. A portion of the specimen was inoculated on one plate each of the Bordet-Gengou and Löwenstein media, also on a blood plate and in blood broth in order to detect other organisms. If there was no growth after forty-eight hours' incubation, several more plates of the Bordet-Gengou and Löwenstein media were inoculated with the remainder of the specimen which was kept refrigerated. If the specimen showed contamination on the preliminary cultures, it was treated with oxalic acid in the manner described for sputa.

Pleural Fluids, Spinal Fluids, and Other Internal Effusions.—These were usually condensed by centrifugation for one hour at high speed. The sediment was examined by smear, and, if the result was negative, they were cultured as described under "Pus."

Tissues.—The surface of the tissue was seared and the infected areas were removed and macerated with sterile scissors. A heavy suspension of the tissue was made in sterile distilled water by grinding it in a sterile mortar and gradually adding the water. The suspension was filtered through sterile cotton to remove large particles of tissue and was tested for contaminants as described under "Pus." If contaminants were present, the remainder of the suspension was treated with an equal amount of 4 per cent sodium hydroxide* and was incubated at 37° C. for forty-five minutes. The reaction was then adjusted to pH 6.8-7.0 with 10 per cent hydrochloric acid and cultured on several plates of Bordet-Gengou and Löwenstein media.

Stomach Contents and Urine.—The specimens were centrifuged at high speed for one hour. The sediment was suspended in sterile distilled water and treated the same as sputum.

Throat Swabs.—The swab was placed in a tube containing a small amount of sterile distilled water, and an equal amount of oxalic acid was added. The tube was shaken vigorously and incubated at 37° C. for forty-five minutes. The liquid was filtered through sterile cotton and cultured.

We have cultured in this manner 1,108 specimens and the results are summarized in Tables I and II.

TABLE I

SPECIMENS NEGATIVE ON SMEAR EXAMINATION

SPECIMEN	TOTAL CULTURED	BORDET-GENGOU MEDIUM				LÖWENSTEIN MEDIUM				TOTAL POSITIVE ON BOTH MEDIA	
		POS.	NEG.	CONTAM.	% POS.	POS.	NEG.	CONTAM.	% POS.	NO.	%
Sputum	716	82	590	44	11.4	73	617	26	10.1	96*	13.4
Tissues	109	26	78	5	23.8	21	84	4	19.2	27	24.7
Pus	39	19	19	1	48.7	21	18	0	53.8	23	58.9
Pleural fluid	149	61	85	3	40.9	61	88	0	40.9	72	48.3
Spinal fluid	6	3	2	1	50.0	4	2	0	66.6	4	66.6
Miscellaneous fluids†	8	0	7	1	0	0	8	0	0	0	0
Stomach contents	14	0	11	3	0	2	10	2	14.2	2	14.2
Throat swabs	8	0	8	0	0	0	8	0	0	0	0
Urine	59	8	45	6	13.5	6	49	4	10.1	10	16.9
Total examined	1,108	199	845	64	17.9	188	884	36	16.9	234	21.1

*There were some positive on one medium and negative on the other. Therefore, the total number of positive is greater than on either one of the media.

†Abdominal fluid, knee fluid, bursitic fluid, scrotal fluid, hydrocelic fluid.

The largest percentage of positive results was obtained on culturing pus, pleural fluids, and spinal fluids, followed, in the order given, by tissues, urines, and sputa (see Table I). If one takes into consideration that all of these specimens were negative on smear examination, the results seem highly satisfactory.

Growth of tubercle bacilli appeared on both media within two to three weeks; the average period of incubation was seventeen to eighteen days. In a few instances, growth appeared in nine to ten days. Occasionally when the growth was very scant—one to three colonies on all the plates inoculated—they

*We have changed recently to 5 per cent oxalic acid with equally good results.

were not detected until four or five weeks of incubation. For this reason, no specimen was reported negative until the culture was incubated five to six weeks. A positive result was reported to the physician as soon as typical growth appeared, which was confirmed by smear examination. When the growth was atypical but the smear of the colonies showed acid-fast bacilli, a preliminary report was sent to the physician and a final report was made after the culture was tested for virulence. All cultures of acid-fast bacilli that were obtained from urine were tested for virulence, although most of them were typical on colony morphology.

The growth of tubercle bacilli on the Bordet-Gengou medium was, in many instances, detected earlier than on the Löwenstein medium. The young colonies on the Bordet-Gengou medium are pearly in appearance and stand out more clearly against the dark background of the medium.

TABLE II

COMPARISON OF THE GROWTH ON THE BORDET-GENGOU AND THE LÖWENSTEIN MEDIA OF 234 POSITIVE CULTURES

SPECIMENS	BORDET-GENGOU MEDIUM	LÖWENSTEIN MEDIUM
153	Positive	Positive
36	1 to 10 colonies	Negative
10	Fairly good growth	Negative
18	Negative	1 to 3 colonies
1	Negative	Fairly good growth
16	Contaminated	Positive*

Positive on the Bordet-Gengou medium	199
Positive on the Löwenstein medium	188

*Six of these specimens had 1 to 4 colonies, 1 had scant growth, and 9 had good growth.

Comparing the results obtained with the two media, we find that more specimens were lost through contaminations on the Bordet-Gengou than on the Löwenstein medium (Table I). This is not surprising since the Bordet-Gengou medium has no inhibitory substances, whereas the Löwenstein medium contains a dye which restricts the growth of some of the contaminants. However, in spite of the greater loss of specimens through contaminations, the total number of positives on the Bordet-Gengou medium was greater than on the Löwenstein medium (see Tables I and II). If the pathologic material for culture could be obtained without undue contamination, or if the specimens could be cultured soon after they are obtained, the Bordet-Gengou medium could be used alone with excellent results.* We cultured 279 miscellaneous specimens from October, 1937, through December, 1938, and of this number we lost only 6 specimens through contaminations.

The use of both media had the advantage that in some instances growth was obtained on the Löwenstein medium when the Bordet-Gengou medium was negative or contaminated (Table II). Of 81 specimens that were positive on only one of the two media, 46 were positive on the Bordet-Gengou and negative on the Löwenstein medium, 19 were positive on the Löwenstein medium and negative on the Bordet-Gengou medium, and an additional 16 specimens were positive on the Löwenstein medium when the Bordet-Gengou medium was over-

*Gernez, Crampon, and Graux[9] confirmed our findings with regard to the value of the Bordet-Gengou medium in the isolation of human and bovine tubercle bacilli.

grown by contaminants. Thus an additional 35 specimens were found positive by using the Löwenstein medium in addition to the Bordet-Gengou medium. It would, therefore, be advisable to use both media if they are available. The use of more than one medium has been recommended by Whitehead[10] recently when he tested several different media. He found that Petragnani's medium gave much better results than the Löwenstein medium. In our hands, the results on both media were approximately the same.

We found the Bordet-Gengou medium especially advantageous because it is an inexpensive medium and both the human and bovine types of tubercle bacilli grow well on it. It is easily prepared and can be kept in the icebox almost indefinitely if it is stored without adding the blood. It may be stored for several weeks when the blood is added, but after that it tends to become dry and is less favorable for the growth of tubercle bacilli. This medium was prepared according to the original formula[4] until about one year ago, when we began using the modification of the Bordet-Gengou medium that was recommended by the Committee of Standard Methods of the American Public Health Association.[11] We found that the growth of tubercle bacilli on the modified Bordet-Gengou medium was more luxuriant than on the original medium provided approximately 30 per cent of normal blood was added to the medium. We use normal horse blood because it is readily available in our laboratories.

The Löwenstein medium was prepared according to the original formula.[5] The only change made was that we use 1.5 per cent malachite green instead of 2 per cent Congo red.

SUMMARY

Cultural diagnosis of tuberculosis instead of guinea pig inoculation has been employed in our laboratories for the past five and one-half years. Only those specimens were cultured that would ordinarily have to be injected into guinea pigs, that is, those that could not be diagnosed on smear examinations.

A total of 1,108 specimens of sputa, tissues, pleural fluids, pus, urines, and other pathologic materials were cultured. Each specimen was examined by smear, and those that were negative were cultured on the Bordet-Gengou and Löwenstein media.

The largest percentage of positive results was obtained on culturing pleural fluids, spinal fluids, and pus—48.3 to 66.6 per cent. Tissues were positive in 24.7 per cent, sputa in 13.4 per cent, and urines in 16.9 per cent.

Positive cultures were obtained within two to three weeks, with an average of seventeen to eighteen days. Occasionally a positive culture was obtained in nine to ten days, and in a few instances, where the growth was very scant, it was not detected until the culture was incubated for four to five weeks. If the cultures were negative after five to six weeks' incubation, no growth was obtained on longer incubation. We, therefore, made the period of incubation five to six weeks.

More positive cultures were obtained on the Bordet-Gengou than on the Löwenstein medium, but occasionally growth of tubercle bacilli was obtained on the Löwenstein medium when the Bordet-Gengou medium was negative or overgrown by contaminants.

CONCLUSIONS

1. Cultural diagnosis of tuberculosis on the Bordet-Gengou and Löwenstein media has been found practicable, and the results obtained compare favorably with the results that we obtained formerly on guinea pig inoculation.

2. The Bordet-Gengou medium has the special advantage that it is inexpensive. It can be kept for a long time, and tubercle bacilli of both the human and the bovine type grow well and characteristically on it. Its main disadvantage is that it has no inhibitory action on contaminants that resist acid or alkali treatment. It is, therefore, advisable to use another medium that has inhibiting substances in conjunction with the Bordet-Gengou medium. The Löwenstein[5] medium has been used with good results. If the pathologic material could be obtained without undue contamination, the Bordet-Gengou medium could be used alone with excellent results.

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COVER SLIP DISPENSER*

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THE machine illustrated in Fig. 1 was designed as an aid in handling cleaned cover slips. The cover slips are ejected singly in a position to be easily grasped by the edges without smearing the cleaned surfaces.

In essence the machine is composed of a plunger of correct thickness, running on a horizontal plane through a box containing cover slips, the edges of the box being raised sufficiently in the direction of motion of the plunger to allow only one cover slip to be pushed out of the box by the forward motion of the plunger. From the illustration, the construction can be easily followed.

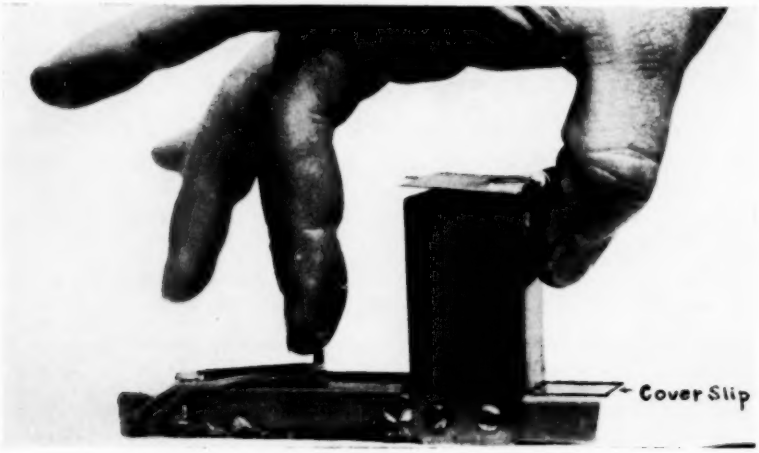

Fig. 1.

The box holding the cover slips can be cut out and bent to shape from scrap pieces of light gauge stainless steel or monel metal from a tinner's shop. The edges under which the plunger and cover slip run must, of course, be parallel with the base. The bolt holes in the box should be elongated in order that the slit for the emerging cover slips can be adjusted to the correct height. The inside dimensions of the box must not be much larger than the cover slip, but the height is a matter of choice. Not visible in the illustration is an angled extension of one side of the box, about two-thirds of the height from the base, to facilitate insertion of the cleaned slips. A hinged cover is attached to the box to protect the slips from dust.

The base must be rigid and conform to the size of the box. It is perhaps best made of a piece of channel bar of the correct size. The top surface of the base must be square and smooth.

*From the Laboratory of the C. V. Memorial Hospital, Johnstown.
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The plunger, of which only the knob is visible, is the 0.006 inch blade of a thickness gauge, soldered at the back end to a heavier piece of metal. The front of the blade, the width of the box, must be free to slide through the box. The blade must be sufficiently long and not too pliable. The heavier block lends rigidity to the plunger and is arranged so that its front edge against the box acts as a stop to forward motion. A screw in the proper place in the back of the base is a stop to backward motion. On the heavier block is soldered a knob to facilitate moving the plunger.

The runway is merely two pieces of angle adjusted to fit the heavier block of the plunger and soldered on the base. The solder can doubtless be applied more smoothly than that shown in Fig. 1. There should be comparatively little horizontal or vertical play in the plunger.

A pin is soldered on either side of the back and the base as an anchor for a spring (rubber band) which returns the plunger to position for another stroke.

By adjusting the slit at the base, either No. 1 or No. 2 cover slips can be dispensed.

THE IDE TEST FOR SYPHILIS

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FOLLOWING the publication of a report on a simple test for syphilis, as devised by Dr. T. Ide and Dr. S. Ide[1] in this JOURNAL August, 1936, an attempt has been made to evaluate this test in general practice.

The Ides state that the test has special advantages in that with only one drop of blood and a simple technique the result can be read clearly by the coloring under the microscope. It can be made in a few minutes' time in the examining room. They state that the accuracy of the test compares favorably with the Wassermann, Meinicke, Sachs-Georgi, Kahn, and Murata. The test can be performed with blood serum, whole blood, spinal fluid, and serous exudate.

The desirability of a simple test for syphilis is apparent. A test that can be done quickly, accurately, and inexpensively would be of great value to the practicing physician without a large laboratory. It would certainly be a boon to the physician practicing in the country, who, though having access to laboratories miles away, would find a test of this type exceedingly helpful. The test described herein approaches in simplicity a routine urinalysis.

A few evaluations of this test have appeared in the literature, but the results as given are somewhat conflicting. Most of the reports seem to have come from laboratories where standard serologic tests are also done. Thus, if this test is of value, its agreement with standard tests should be higher than the

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results obtained by one who is untrained in serologic work. The test as outlined, however, is for the benefit of the latter worker as well as for serologic experts.

The *British Medical Journal*,[2] in an editorial, state that the test seems worthy of trial. They raise the warning that it is doubtful whether a serologic test for syphilis is yet sufficiently foolproof to be removed from the hands of the experienced pathologist. They point out that even the simplest tests have pitfalls, not only in the setting up of the reaction, but also in the reading and interpretation of the results.

In 1,262 tests the Ides[1] obtained 1,126 negative Wassermann reactions, and 1,140 negative Ide tests. One hundred nineteen persons gave strongly positive Wassermann reactions, and 116 gave strongly positive Ide reactions. They obtained no false positive Ide tests.

Rein and Hazay[3] have modified the Ide test by inactivating sera at 56° C. for thirty minutes. They have also used slides similar to those used in the Kline test. Of 482 nonsyphilitic persons whose sera gave negative reactions with the Kline diagnostic and exclusion test, 92.11 per cent gave negative reactions with the Ide test. Of 555 syphilitic sera, there were 2.88 per cent disagreements. They conclude that the Ide test has been found to be somewhat less sensitive and less specific than the Kline heated serum tests.

Flood and Moyer[4] found that 51 per cent of the Ide tests agreed with the standard tests performed on 565 specimens of syphilitic sera. Of the total 822 Ide tests they performed, there was agreement with controls in 61 per cent. They are at a loss to explain these results. They conclude that the Ide test is inadequate for the serodiagnosis of syphilis.

Breazeale, Greene, and Harding,[5] in a large series of cases (1,000), state that the Eagle, Ide, Kahn, Kline, and Laughlen tests are equally reliable if the user adheres to the recommended technique.

Demanche and Segol[6] believe that the Ide test is less sensitive than the Wassermann, Kahn, and Meinicke, and is less precise. They believe that the test should be checked by more exact methods. (Series 295 cases.)

Our report is based on 210 tests carried out in a small office, with no special laboratory space. Routine blood counts, urinalyses, and smears are about the only laboratory procedures regularly done in the office. Equipment necessary to conduct the test is inexpensive or already in the physician's possession.

We assembled two 1 c.c. pipettes, graded in hundredths of a cubic centimeter, two hanging-drop slides, cover slips, medicine droppers, the antigen, a small test tube for the diluted antigen, and small quantities of 2.5 per cent and 3.5 per cent saline solution. We used a microscope for reading the test.

The procedure used has been outlined in the original article and has been used on whole blood, serum, and spinal fluid. The majority of the tests have been carried out, using serum which has been separated from the cellular elements either by centrifuging or by standing.

The antigen used was purchased commercially or used from a supply sent by the Ides. The more recent tests have frequently been carried out using both antigens. The antigen has been kept at room temperature. Although

the method for preparing the antigen is described, its preparation seems too involved and time-consuming for the individual who might best use the test.

In brief, the procedure of the test has been to prepare the diluted antigen (0.2 c.c. of stock antigen with 0.6 c.c. of 2.5 per cent saline solution) immediately before it is to be used. When we have had only a very few tests to make at one time, we have used 0.1 c.c. of antigen to 0.3 c.c. saline, with no variation in the results when checked by the full quantity of antigen and saline solution.

A drop of whole blood is placed on the hanging-drop slide and mixed with a drop of 3.5 per cent saline solution, and then spread over the depression. To this is added a drop of the diluted antigen. If blood serum or spinal fluid is used, the dilution with saline is omitted. The slide is then shaken so as to thoroughly mix the antigen and the blood or serum. This should be read at once. We have not used cover slips on the preparations as they seem to be in the way, and the amount of drying and evaporation is slight during the time necessary to mix and read the preparation.

A total of 210 Ide tests have been made and checked against Wassermann, Kahn, and Kline tests. The majority of the Wassermann and Kahn tests have been made in the laboratories of the Knoxville General Hospital or in the laboratories of the Bureau of Health of Knoxville. A few tests have been made by private laboratories. Sera have been obtained from these laboratories following the performance of these standard tests, and the Ide test then made. I have adhered closely to the technique originally outlined as the test could hardly be simplified. The results obtained are shown in Table I.

TABLE I

Ide	Negative	Wassermann	Kahn	Kline	Negative
168		86	75	6	
Ide	Positive	Wassermann	Kahn	Kline	Positive
24		17	6	1	
Ide	Negative	Wassermann	Kahn	Kline	Positive
8		5	3		
Ide	Positive	Wassermann	Kahn	Kline	Negative
8		4	4		
Ide	Doubtful	Wassermann	Kahn	Kline	Doubtful
2		1	2		

SUMMARY

1. The Ide test for syphilis is considered chiefly from the standpoint of the physician without extensive laboratory facilities.

2. A brief review of the literature is given.

3. The results of 210 Ide tests as compared with an equal number of standard serologic tests are given. Total agreement was 92.39 per cent. The number of false positive Ide tests was 4.57 per cent. The number of false negative Ide tests was 25 per cent. Eight negative reactions in a total of 32 positive Wassermann or Kahn reactions are a large margin of error. I do not believe the test sufficiently sensitive and specific to be wholly relied upon for the serodiagnosis of syphilis.

I am indebted to Dr. Thomas B. Drinnen for his assistance in part of this work.

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THE CONCENTRATION OF TUBERCLE BACILLI FROM SPINAL FLUID BY MEANS OF CHEMICAL FLOCCULATION AND LIPOID SOLVENTS*

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WE HAVE shown that the collection of tubercle bacilli from aqueous suspensions, sputum,[1] or urine[2] by direct centrifugation is much less efficient than might be expected in view of the relative ease with which other bacteria are sedimented by this method. For example, after centrifugation of slightly positive sputa for one hour, chemical flocculations revealed that many bacilli remained in the supernatant fluids. The incorporation of 0.2 per cent alum in the usual 4 per cent sodium hydroxide digestor for sputum, and flocculation of the alum by neutralization prior to centrifugation for five minutes, increased the effectiveness of concentration from sputum.

In view of the importance of concentration methods in the diagnosis and control of tuberculosis, these studies have been extended to an examination of the collection of tubercle bacilli from spinal fluids by means of several of the methods in current use, and especially by means of chemical flocculation and lipoid solvents.

METHODS

Two kinds of material were studied: a series of "synthetic" tuberculous spinal fluids prepared for the study of various technical problems, and a series of spinal fluids submitted for bacteriologic confirmation of the clinical diagnosis from persons suspected of tuberculous meningitis.†

The synthetic tuberculous spinal fluids‡ were prepared by addition of known amounts of clump-free, precounted suspensions of human type (H-37)

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†We are indebted to the Clinical Laboratory, Dr. E. Clarence Rice, and the resident staff of the Children's Hospital, for these specimens and for information from the hospital records of each case.

‡The usefulness of such materials in studies of this nature has not been sufficiently appreciated. The bacillary content of the "synthetic" tuberculous spinal fluid is known, and a measure of the actual degree of concentration may be obtained by quantitative counting. The suspension, free of clumps, provides the most rigorous test for the collecting power of any method. At the same time one avoids the errors introduced by the chance distribution and disruption of clumps. The "synthetic" fluids can be prepared in amounts which permit the simultaneous testing of any number of methods. By means of this experimental approach it is possible not only to select the better methods but also to indicate the increased possibility of demonstrating the bacilli by a *concentration factor.*

tubercle bacilli to pooled normal spinal fluids. The method of suspending the bacilli, the separation of the individual bacilli from the clumps, and the preliminary standardization by turbidity, have been given elsewhere.[3] These suspensions were heated at 80° C. for fifteen minutes in the presence of 0.2 per cent formaldehyde. This procedure did not alter the microscopic counts. The heated suspensions were stored in the icebox for several weeks without loss of acid-fastness and with negligible clumping. Nevertheless, each suspension was counted prior to its use in each experiment.

The microscopic method of counting the bacilli consisted of mixing the bacillary suspensions, or the sediments after concentration, with an equal volume of alum milk (Difco dehydrated milk 5 per cent, alum 0.5 per cent, formaldehyde 0.2 per cent) and spreading 0.02 c.c. of this mixture on carefully standardized areas of 2 by 2 cm. on clean glass slides in duplicate. The films were dried, fixed with heat, and stained by immersion in Ziehl-Neelsen carbolfuchsin at 37° C. for thirty minutes. Staining, decolorization, washing, and counterstaining (methylene blue or picric acid) were carried out in jars to avoid the slide losses induced by the action of running water on the films. The stained films were examined by counting the bacilli in ten or more fields selected in orderly fashion along each diagonal of the films, so that 40 or more fields were observed in each determination from the two films. Data on the distribution of bacteria in such films and on the quantitative aspects of microscopic counting will be presented elsewhere.[4]

In the various experiments the original suspensions revealed from two to six bacilli per microscopic field before being added to the pooled spinal fluids in such amounts that the final dilution was 1:100. The number of bacilli which might be anticipated per field in the unconcentrated fluids (0.02 to 0.06 bacilli per field) was regarded as unity. The average number of bacilli counted per field after concentration represented a multiple of the value of unity in each experiment. This multiple, to be designated as a *concentration factor,* indicates the degree of concentration. It affords a simple means of comparing the effectiveness of different methods. All clumps were scored as single bacilli.

For each experiment a sufficient amount of the "synthetic" tuberculous fluid was prepared to provide 2 c.c. portions to be concentrated by each procedure. These tests were carried out in small tubes in order to simulate the conditions of manipulation and centrifugation encountered in small clinical specimens. The methods of concentration are classified and summarized as follows.

A. *Centrifugation:*

1. Cheer's alcohol method[5]—Addition, by drops, of one-third to one-half volume of alcohol and, if needed, a small amount of egg albumen to produce precipitation. Centrifugation as described under Direct centrifugation.
2. Direct centrifugation—Centrifugation for one hour at a relative centrifugal force of 500 times gravity; sediments smeared in approximately equal volume of alum-milk fixative.

These procedures are widely used at the present time and, in this sense, may be regarded as controls.*

B. *Flocculation Methods:* Centrifugation for five minutes; sediments mixed with an equal volume of alum-milk fixative.

1. Alum—The 2 c.c. specimen was pipetted forcibly into 0.25 or 0.5 c.c. of 1 per cent aqueous solution of potassium alum. Flocculation was prompt but slight.
2. Calcium phosphate—0.1 c.c. M/10 calcium chloride and then 0.1 c.c. M/15 dipotassium phosphate were added to the fluid during constant shaking.
3. Ferric chloride—1 per cent aqueous solution of ferric chloride was added drop by drop (with continuous shaking) until flocculation was observed.

C. *Lipoid Solvents:* Centrifugation for five minutes; the collected materials could not be mixed with milk fixative.

1. Chloroform—0.05 c.c. was added to each 2 c.c. specimen. The tube was shaken violently for ten minutes; the entire sediment beneath the discarded supernatant was used.
2. Xylol—Procedure as above; the supernatant creamy layer was used.

RESULTS

Inasmuch as the studies in the "synthetic" fluids revealed objectionable features in several of the foregoing methods, these difficulties will be considered first in order to limit the discussion to the more useful procedures.

Cheer's method was more troublesome than direct centrifugation in that several alternate additions of alcohol and egg albumen were required. The results were frequently inferior, perhaps because of the dilution of the sample by the reagents. It has been noted that the collection time for each of the centrifugation methods was one hour.

The calcium phosphate method was disqualified because two separate reagents were required and because the sediments did not smear well when used alone or with the milk fixative. Sudden, voluminous precipitation occurred frequently with ferric chloride. The large sediments interfered with effective concentration. Furthermore, the 1 per cent solution of ferric chloride deteriorated on storage.

The xylol flotation method apparently collected fewer bacilli than the chloroform, possibly because the xylol cream could not be skimmed from the centrifuged sample with ease or completeness. A satisfactory rating could not be assigned. Since the chloroform sediments from a clear solution, such as spinal fluid, were not objectionable because of debris, smeared well, and could be recovered completely from the tubes after removal of the supernatant fluid, this method was preferred to the xylol flotation. In the case of either lipoid solvent, the use of milk fixative was not feasible and, apparently, not

*Isolated observations on the possibility of demonstrating tubercle bacilli in the "web" have been discouraging.

necessary. A peculiarity of the lipoid solvent methods was that the collected materials could not be smeared if collection was made from formalinized spinal fluids in the neutral or alkaline range of pH values. This difficulty was overcome by the addition of two drops of bromcresol green color indicator and sufficient N/1 hydrochloric acid to produce a green or yellow color (pH approximately 4.5 to 4.0).

In Table I are shown the average concentration factors for the two most satisfactory methods (alum and chloroform) and the control method of direct centrifugation. The values are based on four of the experiments in which the most reliable comparisons were obtained.

TABLE I

AVERAGE CONCENTRATION FACTORS FOR THREE IMPORTANT METHODS

METHODS	CENTRIFUGATION TIME	CONCENTRATION FACTOR	REMARKS
Centrifugation	1 hour	x25	
Alum	5 minutes	x92	To be recommended for cultivation
Chloroform	5 minutes	x102	To be recommended for microscopic work

On the basis of simplicity and effective concentration, the alum and chloroform methods were superior to all others. There was no significant difference in their collecting ability from "synthetic" tuberculous fluids free of clumps of bacilli. In the meantime, however, a study of the specific problems encountered in spinal fluids from tuberculous meningitis indicated that each procedure may serve a special purpose, depending on whether diagnosis is to be carried out by microscopic methods or by cultivation and animal inoculation.

The great frequency with which clumps occurred in clinical specimens is illustrated in Table II, which shows detailed records of the distribution of bacilli collected by flocculation methods from four of the spinal fluids from tuberculous meningitis. These examples reveal that more than 200 bacilli were seen in clumps while only 13 were encountered as individual bacilli. Similar observations have been made by other workers; for example, Boyd[6] states that ". . . . the bacilli are gathered into little clumps as in the urine. . . ."

TABLE II

THE FREQUENCY OF BACILLARY CLUMPS IN SPINAL FLUIDS FROM TUBERCULOUS MENINGITIS AND THE POTENTIAL ADVANTAGE OF DISTRIBUTING THESE BACILLI

FIELDS POSITIVE	NUMBER OF CLUMPS AND SINGLE BACILLI IN EACH POSITIVE FIELD*	BACILLI IN CLUMPS	BACILLI SINGLE	POTENTIALLY† POSITIVE FIELDS
2/108	c8, c14	22	0	1 in 5
5/170	1s, 1s, 1s, 1s, c>50	>50	4	1 in 3
7/60	c8, 1s, 2s, c3 and 3s, c5, 2s, c12	28	8	1 in 2
3/20	1s, c4, c>100	>105	1	100 per cent

*c8 = clump of 8 bacilli; 3s = 3 single bacilli.

†In clinical specimens calculations of the potential incidence of positive fields do not prove that each bacillus liberated from a clump will produce a positive field. Nevertheless, the figures reveal that much could be gained by distributing the bacilli which so frequently occur in clumps.

Incidental observations on the clinical samples also suggested that chloroform concentrates contained fewer bacilli as clumps than did centrifuge or alum sediments. Since the alum and the chloroform methods were approximately

equal in their ability to collect unit masses of bacilli, their action on clumped suspensions was compared. "Synthetic" tuberculous spinal fluids were prepared from bacillary suspensions which, when examined microscopically as hanging drops, revealed frequent small clumps. The tubes containing the alum precipitation samples were also shaken for ten minutes according to the routine method for the chloroform procedure. The concentrates prepared by each method were divided into two parts. One was smeared directly and the other was subjected to grinding for three minutes before being smeared. It was observed that during the shaking period the chloroform was entirely taken up in the sample. An additional 0.05 c.c. was added before recovery of a chloroform concentrate was possible.

TABLE III

COMPARISON OF THE CLUMP-BREAKING ABILITY OF ALUM AND CHLOROFORM, AS MODIFIED BY MECHANICAL GRINDING OF THE CONCENTRATES

METHOD	GRINDING FOR THREE MINUTES	POSITIVE FIELDS	BACILLI IN CLUMPS	BACILLI SINGLE	TOTAL BACILLI
Alum	−	6/20	65	6	71
	+	8/20	39	5	44
Chloroform	−	18/20	0	61	61
	+	19/20	0	68	68

The results summarized in Table III indicate that, in spite of clumpy distribution, there was a reasonable agreement in the total number of bacilli collected. Nevertheless, the shaking and the mechanical grinding of the concentrates did not distribute the clumped bacilli collected by the alum procedure. These observations, together with the increased requirement for chloroform, indicate that this reagent itself was responsible for the disruption of the clumps. Pottenger's earlier conclusions[7] concerning the action of lipoid solvents on clumps of bacilli in clinical specimens were amply confirmed.

The examination of the spinal fluids from tuberculous meningitis was carried out whenever material was available during the course of the study. Consequently, the flocculation and lipoid solvent procedures were modified several times in order to check the results of the experimental work or to improve the results in the diagnostic trials. Because of the small amounts of spinal fluid, we hoped that the results of centrifugation in the hospital laboratory could be compared with those obtained by the concentration methods we studied. Unfortunately, such a comparison was not justified on account of differences in the staining quality of the carbolfuchsin employed in the two laboratories.

Thirteen spinal fluids from 11 persons proved to have tuberculous meningitis were examined by one or more of the methods investigated. Two of these were examined by culture only and were positive. One was negative microscopically but positive on cultivation from the alum sediment. Among the 10 fluids remaining, 7 were positive by one or more of the flocculation or lipoid solvent methods. Of the 3 microscopically negative specimens, 2 fluids were from persons who gave microscopically positive results in another examination (these two repeats account for the 13 fluids from 11 persons); one fluid was

obtained from a patient *in extremis* and further examination of this person was not possible. At necropsy the entire brain was found to be encased in a gelatinous exudate which covered the tubercles in the meninges.

In one spinal fluid, which was positive by direct centrifugation also, a comparison with the results obtained by the use of chloroform was interesting. Forty-three fields were examined in smears obtained by each method. In the sediment obtained by centrifugation, one positive field contained two bacilli, while in the chloroform concentrate there were 12 positive fields. The observations in these fields are presented by the system employed in Table II, as follows: 8s, 3s, 1s, 1s, 1s, 1s, c6, 1s, 1s, 3s, 1s, 1s, or a score of 23 bacillary units in 43 fields.

It must be emphasized that, although the observations in the "synthetic" fluids were supported by the results in the clinical materials, the latter did not provide an adequate means of assessing the relative value of collection methods. The amounts of material were too small for testing all the methods, and the bacillary content was too low and variable to permit significant comparisons. For the present, the experimental work constitutes the chief justification of this report.

DISCUSSION

Although the clinical specimens were too few to justify further comment on practical results, the work with alum and chloroform in the experimental trials showed that these procedures consistently collected more bacilli than Cheer's alcohol method or direct centrifugation; the average *concentration factors* were approximately four times higher.

The alum and the chloroform methods collected unit masses of bacilli equally well, but the chloroform was shown to be definitely superior in disrupting the clumps which occur so frequently. This method produces more positive fields and is suggested for microscopic diagnosis. The procedure recommended for qualitative purposes is as follows:

Chloroform Method for Microscopic Diagnosis

1. To each 2 c.c. of spinal fluid, add 0.05 c.c. (or 4 drops) of chloroform and shake violently for ten minutes. See special instructions for formalinized fluids.
2. Centrifuge at top speed for five minutes; discard supernatant fluid.
3. Smear entire sediment without added fixative, heat fix, stain with carbolfuchsin, and decolorize. Use methylene blue or picric acid counterstain according to the density of the smears.

The passage of acid-fast bacteria from water to oils and lipoid solvents is well established. References and recent information appear in a publication by Reed and Rice.[8] Ligroine,[9] xylol,[10] and chloroform[11] have been used in concentration methods for sputum. Pottenger's data[7] on the resolution of bacillary clumps by lipoid solvents were confirmed in our experience with chloroform.

Inasmuch as the presence of clumps is perhaps favorable to cultivation or animal inoculation, and since practically the same number of bacilli *in toto* are collected by alum and by chloroform, it was concluded that the alum concentrates should be chosen for cultivation or animal inoculation. The recommended procedure follows:

Alum Flocculation Method for Cultivation or Animal Inoculation

1. Pipette the specimen forcibly into an autoclaved 1 per cent aqueous solution of potassium alum* (0.25 c.c. alum per 2 c.c. of fluid). Shake immediately for ten minutes.
2. Centrifuge for five minutes; discard supernatant fluid.
3. Cultivate the sediment on 3 to 5 tubes of suitable medium or emulsify and inject into guinea pigs.

For microscopic purposes the alum concentrates are superior to sediments obtained by direct centrifuging for one hour. They should be smeared in a very small drop of the alum-milk fixative and stained as previously indicated.

SUMMARY AND CONCLUSIONS

1. A study of the bacteriologic diagnosis of tuberculous meningitis by means of chemical flocculation and by lipoid solvent methods showed that alum and chloroform provided more effective concentration of the bacilli during five minutes' centrifugation than was obtained by direct centrifugation for one hour.

2. For microscopic diagnosis it was important to distribute the clumped bacilli as well as to obtain efficient concentration; for this purpose the chloroform procedure was superior.

3. The alum flocculation method is recommended for cultivation or guinea pig inoculation.

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*A film is formed on the tube in which the alum is autoclaved, but the effectiveness of the solution is not impaired.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TYPHOID FEVER, Intracellular Bacilli in Intestinal and Mesenteric Lesions of, Adams, J. W., Jr. Am. J. Path. **15**: 561, 1939.

Gram-negative, intracytoplasmic bacillary forms, judged to be *E. typhi*, have been found in the cytoplasm of young plasma cells located in the lymphoid follicles of the ileum, colon, and mesenteric lymph nodes in 5 patients with early typhoid fever.

A method for staining intracellular bacteria is described. It is concluded that the presence of these bacillary forms within the plasma cell is an essential part of the early, classical, intestinal and mesenteric lesions of typhoid fever.

Method:

1. Stain in Weigert's iron-hematoxylin solution for one minute or less.
2. Wash in 50 per cent alcohol, acidulated to 0.1 per cent hydrochloric acid.
3. Stain in Goodpasture's carbol-aniline-fuchsin solution for one minute.
4. Decolorize in a 5 per cent aqueous solution of acetic acid until the red color disappears or the sections remain a light pink.
5. Repeat steps 3 and 4 three times.
6. Wash in water.
7. Stain in a 0.01 per cent light green solution, acidulated to 0.2 per cent acetic acid, for about one minute, or until the section has a light green hue. Avoid overstaining.
8. Wash in water, differentiate in 95 per cent alcohol, dehydrate in absolute alcohol, clear in xylol, and mount in balsam.

The nuclei stain black; the nucleoli, red; cytoplasm, pale green; bacteria, brilliant red; red blood cells, red; other structures, green.

PANCREAS, Islet Cell Tumors of, Campbell, W. R., Graham, R. R., and Robinson, W. L. Am. J. M. Sc. **198**: 445, 1939.

Four cases of islet cell tumor of the pancreas and one case of diffuse hyperplasia of islets with hypertrophy of islet cells are presented.

A varied clinical symptomatology, dependent on the hypoglycemia, is shown, which parallels the results of experimental hyperinsulinism in animals. From the mildest of isolated episodes, the attacks run a course eventually resulting in marked mental deterioration and degeneration of the nervous system; marked obesity occurs as the disease progresses. Thus early diagnosis and adequate treatment are especially desirable, not only to prevent the unfavorable later results, but also to avoid the increased technical difficulties encountered in later operation. While in mild diffuse hyperinsulinism dietary treatment may suffice and the patient may revert to normal, the process in tumor cases is progressive, and nothing short of excision of the tumor will give the patient relief from his symptoms. The islet cells in these tumors exceed the mass of islet cells in the normal pancreas, and their insulin content is from 4 to 40 times that of the normal pancreas per gram, but not in toto. The clinical symptoms seem largely dependent upon uncontrolled secretory activity of the tumor rather than upon continuous production of excess quantities of insulin until the later stages, when the latter factor plays a larger role.

CL. WELCHII, Observations on a Reaction Between the Lethal Toxin of (Type A), and Human Serum, Nagler, F. P. O. Brit. J. Exper. Path. **20**: 473, 1939.

A reaction is described between the lethal toxin of *Cl. welchii* (type A) and the normal human serum in which the serum becomes opalescent. When the opalescent serum is centrifuged, a fatty material rises to the surface.

The reaction is specifically inhibited by *Cl. welchii* antitoxin.

The reaction can also be observed if *Cl. welchii* of any type (A, B, C, or D) is grown in a mixture of normal human serum and broth. This cultural reaction has been shown to be specific for *Cl. welchii* and specifically inhibited by *Cl. welchii* antitoxin.

The lethal toxin of *Cl. welchii* (type A) can be titrated by means of the reaction, the activity of the toxin in bringing about the change in human serum running parallel to its toxicity for mice.

Cl. welchii (type A) antitoxin can likewise be titrated, the inhibiting action of the antitoxin in vitro running parallel to its neutralizing action in vivo.

Technique.—Cultural reaction in human serum: Three-tenths cubic centimeter of a mixture of equal parts of inactivated human serum and nutrient broth is pipetted into a test tube, of approximately 5 mm. internal diameter. Three-tenths cubic centimeter of the same mixture is pipetted into a similar test tube, to the contents of which is added 1 drop of *Cl. welchii* (type A) antitoxic serum, containing at least 20 units per c.c.

Both tubes are inoculated with 1 drop of culture of *Cl. welchii* and incubated for sixteen hours in an anaerobic jar. While the great majority of strains of *Cl. welchii* grow in pure serum, a small percentage will grow only in the serum-broth mixture. The latter strains do not readily produce a stormy clot in milk, although by all other criteria, biologic and serologic, they are true *Cl. welchii*.

After incubation the tubes are examined. In the tube without antitoxin there is an obvious opalescence, with a fatty layer at the top of the medium. The bacteria are seen at the bottom of the tube, although in some mucoid strains they are suspended in the medium. If doubt exists whether an opalescence in the medium is due to the suspended bacteria or to a change in the medium, centrifugation will clear up the doubt. After centrifugation a fatty layer will be found at the top of the medium, and bacteria at the bottom of the tube. In the tube with antitoxin the medium remains clear, with a sediment of bacteria.

PNEUMOCOCCI, A New Method of Culturing Sputum on Solid Media Using Carbon Dioxide for the Isolation of, Auger, W. J. Brit. J. Exper. Path. **20**: 439, 1939.

A "sputum plate" technique for the isolation of pneumococci is presented.

The stimulating effect of carbon dioxide on respiratory pathogens, particularly pneumococci, has been noted.

A maximum growth of pneumococci in fifteen hours on blood agar plates is possible with this method. The method follows:

The sputum is collected from the nasopharynx in children or from the larynx in adults, using the aspiration method. This latter method ensures that the patient does not salivate instead of expectorate. The sputum is then washed in a Petri dish with about 10 to 20 c.c. of saline, which tends to remove mouth organisms and leave the purulent particles containing the likely pathogenic bacteria. A fresh blood agar plate (not older than eighteen hours) is then inoculated. This culture plate consists of 14 c.c. of 2 per cent nutrient agar, to which is added 1 c.c. of human or sheep's blood. The purulent particle is well stirred in the saline and then picked out, using a straight piece of platinum wire. The size of this particle depends on the appearance of the sputum on direct smear. If there are numerous organisms present, the particle must be correspondingly small, and vice versa. The chief difficulty of the procedure lies in the selection of a suitable particle; it should be no larger than 2 mm. in diameter. If there is any doubt about the particle being representative, the procedure should be carried out in duplicate. This particle is then placed on the center of the plate and spread thinly with a glass spreader. The spreading must be done carefully; the best method is to make two or three initial spreads across the plate in one direction. The spreader is then flamed, and the margins of the initial streak are spread upon the rest of the plate. The glass spreader itself must not be too thin or it will cut the media, and it must be held tightly while stroking. When one is experienced, one-half or one-third of a plate is sufficient for one sputum culture.

A piece of wet blotting paper, about 3 by 3 cm., is then placed on the inner surface of the lid of the Petri dish. The blotting paper should be previously autoclaved and dipped into boiling water to prevent gross contamination of the plate.

The plate is then placed with the blood agar side uppermost in the bottom of an airtight container. In routine work ordinary 1,400 c.c. tin cans, with close-fitting lids, are used, but for more accurate work glass or metal anaerobic jars are needed. Carbon dioxide is then introduced into the vessel containing the Petri dish. This gas is obtained from a commercial cylinder and is led off into a water burette (300 c.c. capacity), and finally introduced into the bottom of the container by means of a rubber tube past the partially opened lid. The actual amount of carbon dioxide introduced does not matter, since it is equally effective over a range of 0.5 to 25 per cent. This means that one should place a minimum of about 100 c.c. of carbon dioxide in a 1,400 c.c. can to allow for leakage past the open lid. After the required amount of carbon dioxide has been introduced slowly into the bottom of the can, the tube is removed and the lid is sealed with adhesive tape. The container must be airtight and then incubated for fifteen hours between 35° and 37° C. Before being used again, it should be washed with warm water.

GLUCOSE TOLERANCE, Analysis of 583 Tests, Watson, B. A. J. Endocrinology 25: 845, 1939.

A study of 583 routine glucose tolerance tests on patients with glycosuria is presented. The incidence of glycosuria in routine urine examinations was 1.14 per cent. No sex difference was noted in the incidence of glycosuria or mean blood sugar values during the tolerance test.

About 15.5 per cent of the patients with glycosuria on routine examination will have evidence of a definitely disturbed carbohydrate metabolism, as indicated by the glucose tolerance test. In this age group the mean blood sugar values during the tolerance test of the overweight group were lower than those of the normal weight group, and the normal weight group were lower than those of the underweight group. Thus, obesity does not appear to predispose a disturbance of carbohydrate metabolism in this age group.

The question of the importance of family history as a predisposing factor to the development of a disturbed tolerance and ultimate diabetes is raised, as subjects with a family history of diabetes had no higher mean blood sugar values during the tolerance test than did those with no such history. Only 17 of the 109 subjects with a family history of diabetes had disturbed tolerance tests. It is noted that there were wide variations in individual responses to the glucose tolerance even in the so-called normal group.

The suggestion made is that routine tolerance tests on patients having glycosuria may be an aid to early diagnosis and treatment of a disturbed carbohydrate metabolism, thus preventing the onset of clinical diabetes.

TUBERCULIN TEST in the Control of Tuberculosis, Doull, J. A. Am. Rev. Tubere. 40: 634, 1939.

It is a significant fact that, although there has been a tremendous decline in mortality from tuberculosis, there is evidence that the risk for household contacts has not been appreciably reduced. The data of Downes, for example, indicate approximately the same mortality rates for those exposed in household and born between the years 1868 and 1897 as for those exposed and born during the period 1904 to 1913. As the disease declines, therefore, it is apparently more and more concentrated in the immediate environment of the open case.

Surely, therefore, the field of action is becoming more sharply delimited. The population primarily at risk is, admittedly, not completely enumerated in any community. But a large proportion of these individuals are listed on health department records, being members of families of those who are known to have open tuberculosis or of those known to have died of the disease.

There is no place for diffuseness in the tuberculosis program. Nevertheless, time and money are being dissipated in many types of indirect effort. Only when a health depart-

ment has isolated in sanatoriums all its known open cases, when all active cases not in the open stage are under medical care, when a high proportion of all household contacts are undergoing periodic medical examination, including examination by x-rays, when a system of financial subsidy is arranged for needy dependents of wage earners who have been attacked—only then should any consideration be given to indirect methods not of established value, such as that under consideration today.

TUBERCULIN REACTORS and Non-Reactors, X-ray Findings in, Douglas, B. H. Am. Rev. Tuberc. **40**: 621, 1939.

In mass surveys the chief consideration should be the finding of active cases of tuberculosis. The finding of evidence of inactive or obsolete lesions is of less importance.

The tuberculin test has long been known to be subject to certain errors due to anergic states that may develop under certain well-known conditions, such as during certain acute infectious diseases and in terminal states of tuberculosis itself.

The x-ray examination has certain very definite limitations. Small lesions may be inaccessible and not seen. Its value is directly related to the experience and ability of the interpreter.

Granting the presence of error, tuberculin has been useful in screening from the many the few who should be further examined by the more expensive x-ray examination.

The tuberculin test is rarely negative in the presence of significant active tuberculosis: 8 out of 500 persons, 7 of whom died.

As surveys are conducted, and ignoring the cost of an x-ray film, examination of everyone in the group can be carried out with less inconvenience to those examined and with less missed cases than by using a preliminary tuberculin screening.

There are several types of x-ray examination being used that seek to bring down the cost factor, fluoroscopy, sensitized paper, and miniature films; all are of some merit but none of them yet sufficiently developed to entirely supplant tuberculin testing on the basis of accuracy or economy.

There is every indication that one or more of these x-ray examination methods will be developed to an acceptable point, and tuberculin will then be used as a diagnostic aid in such problems as the determination of infection in infants and very young children, and in cases of atypical lesions in adults where tubercle bacilli cannot be found.

The final word cannot be said on this problem as yet, but even though our tools are not perfect, we should not be discouraged in using them, standing ready to adopt new and better ones when they shall have been thoroughly tested.

BLOOD VISCOSITY, With Special Reference to Capillary, Arterial (Approximate) and Venous Blood Specimens, Holbrook, A. A., and Watson, M. V. Am. J. M. Sc. **198**: 750, 1939.

The conclusions to be drawn from this study and the reports in the literature may be given briefly as follows:

1. There is no correlation between blood viscosity and color index.
2. There is direct relationship between the number of erythrocytes and blood viscosity.
3. The normal, average, blood viscosity value is about 5.
4. The blood samples taken from a finger tip in which hyperemia has been produced (so-called "arterial" blood) give as reliable results as capillary or venous specimens.
5. There is no correlation between venous pressure within normal limits and blood viscosity.
6. Comparative studies of capillary, arterial, and venous bloods indicate that this method gives entirely satisfactory results.